EFFECTS OF TRANSVERSE, BODILY MOVEMENT OF MAXILLARY PREMOLARS ON THE SURROUNDING HARD TISSUE

A Thesis

by

KRISTINA MARIE CHRISTOPH

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Peter H. Buschang
Committee Members,	Phillip M. Campbell
	Reginald W. Taylor
	Jian Q. (Jerry) Feng
Head of Department,	Larry L. Bellinger

May 2016

Major Subject: Oral Biology

ABSTRACT

Dental tipping and subsequent loss of crestal bone is a negative effect of dentoalveolar expansion, performed using any current non-surgical approach. Purpose: This study was designed to produce buccal translation and evaluate the effects on the buccal alveolar bone. Methods: A randomized split-mouth design was utilized with seven adult male beagle dogs. The experimental side received a custom-fabricated cantilever appliance that produced a translatory force through the maxillary second premolar's center of resistance. The contralateral second premolar control received no appliance. The premolars underwent 6 to 7 weeks of buccal translation, followed by 3 weeks of fixed retention. Tooth movements were evaluated by intraoral, radiographic, and model measurements. MicroCT was used to quantify the buccal bone differences. Bone formation and turnover were assessed using fluorescent labeling, H&E staining, TRAP staining, and BSP immunostaining. Results: The applied force (100 g) expanded (1.4 mm) and minimally tipped (4 degrees) the experimental teeth. Lateral translation produced dehiscences at the mesial (2.0 mm) and distal (2.2 mm) roots. Bone thickness decreased at the apical (~0.4 mm), mid-root (~0.4 mm), and coronal (~0.2 mm) levels. Histological sections showed new bone formation extending along the entire periosteal surface of the 2nd premolar's buccal plate. TRAP staining demonstrated greater osteoclastic activity on experimental when compared to control sections. Conclusions: New buccal bone forms on the periosteal surface during tooth translation, but buccal bone width decreases and crestal bone loss occurs.

DEDICATION

I dedicate this thesis to my mother, Nury, and father, Victor, the two most selfless, hardworking, and wise people I know. Their love of dentistry inspired me to pursue this field. They have been my forever role models, as I continue to learn from them each day. No words can describe how appreciative I am for their endless love and support.

ACKNOWLEDGEMENTS

I would like to thank the members of my committee, Drs. Buschang, Campbell, Taylor, and Feng for their inspiration and support with this project. I would also like to thank Dr. Helder Jacob for his guidance with the appliance and study designs, as well as Dr. Lynn Opperman and Dr. Ke Wang for their guidance with the histology.

TABLE OF CONTENTS

Page

ABSTRACT ii

DEDICATION iii

ACKNOWLEDGEMENTS iv

TABLE OF CONTENT v

LIST OF TABLES vi

LIST OF FIGURES vii

CHAPTER

I INTRODUCTION AND LITERATURE REVIEW 1

Overview 1
Literature Review 3

II EFFECTS OF TRANSVERSE, BODILY MOVEMENT OF MAXILLARY PREMOLARS ON THE SURROUNDING HARD TISSUE 32

Introduction 32
Materials and Methods 35
Results 46
Discussion 50

III CONCLUSIONS 55

REFERENCES 56

APPENDIX A 62

APPENDIX B 66

LIST OF TABLES

TABLE Page

1. Perimeter Gain with Expansion in Mandibular Arch....6

A1. Experiment Schedule....62

A2. Histologic Designations....63

A3. Appliance Breakages....64

A4. Descriptive Statistics....65

LIST OF FIGURES

FIGURE Page

1. Measurement of Center of Resistance 66
2. Appliance Design 67
3. Radiographic Measurements of Tooth Movement 68
4. Intraoral Measurements of Tooth Movement 69
5. Tipping Measurements 70
6. Force Measurements 71
7. Three-Dimensional MicroCT Reconstructions 72
8. MicroCT: Buccal Bone Height Measurements 73
9. MicroCT: Total Tooth Height and Total Root Height Measurements 74
10. MicroCT: Buccal Bone Thickness Measurements 75
11. Fluorescence Imaging 76
12. Fluorescent Imaging and H&E Sections: Comparison 77
13. H&E Sections 78
14. H&E Sections: Bone-like Matrix 79
15. TRAP Sections 80
16. BSP Immunostaining Sections 81

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

OVERVIEW

Transverse maxillary deficiencies and crowding are common presentations among orthodontic patients. Dentoalveolar expansion is often performed to address these transverse problems. A well-documented negative effect of dentoalveolar expansion, performed using any current non-surgical approach, is dental tipping and subsequent loss of buccal alveolar bone.[1,2] This uncontrolled tipping, or tipping of the crown buccally relative to the root, produces a concentrated area of pressure at the most coronal portion of buccal alveolar bone, leading to dehiscence.[1-3] Uncontrolled tipping occurs because the application of force during dentoalveolar expansion is occlusal to the center of resistance of the teeth. To avoid dental tipping, it is necessary to utilize an appliance system that delivers a buccal force at the center of resistance of the tooth, or an equivalent force system. Such a force application results in bodily transverse movement, which evenly distributes pressure along the buccal bone, resulting in less crestal bone loss.[2]

Recent research evaluating orthodontic expansion has shown osteoblastic activity on the periosteal surface of buccal cortical bone after orthodontic expansion.[4] It is hypothesized that by applying pressure more evenly along the buccal surface of alveolar socket, periosteal bone is better able to respond to the mechanical pressures and lay down bone to counterbalance and possibly outweigh bone resorption. However, our knowledge regarding buccal alveolar bone adaptation and apposition is extremely

limited. The goal of this study was to evaluate the effects of buccal translation on the buccal bone in the absence of tipping. By furthering our understanding of bony adaptation to lateral tooth movement, orthodontists will be better able to make treatment decisions that will maintain the health of the periodontium.

This literature review focuses on lateral tooth movement and its biologic responses. First it addresses the etiologic factors requiring buccal tooth movement, aka expansion, and the various modalities used in orthodontics today. Current understanding of the biology of tooth movement will then be discussed and related to force systems and the response of alveolar bone. The final sections will present research pertaining to the deleterious effects of buccal tooth movement, as well as determinants of the body's ability to respond favorably.

LITERATURE REVIEW

Problem

The NHANES III collected data from 1988-1991 that provided weighted estimates of oral health for 150 million individuals. The oral health component highlighted the need for orthodontic treatment. The survey evaluated alignment (i.e. incisor irregularity) and occlusal characteristics. It showed that the percentage of the population with crowding greater than 3 mm, which includes moderate and extreme crowding cases, was 29.6% in the maxilla and 36.1% in the mandible.[5] Crowding was found to be severe in 10-15% of all racial/ethnic groups, with their dental function and social acceptability being affected. Application of the Index of Treatment Need (IOTN) to this data revealed that 57-59% of adolescents would benefit from orthodontic treatment.[5,6]

The NHANES III study also found the prevalence of posterior crossbite involving two or more teeth to be 8.5% between ages 8-11, 7.9% between ages 12-17, and 9.9% between ages 18-50. Brunelle et al. used data from this study to show the relationship between transverse deficiencies and maxillary alignment.[7] Individuals with fair (3-5 mm) or poor (>6 mm) incisor irregularity demonstrated a much higher prevalence of posterior crossbite than subjects with good (1-2 mm) or excellent (0 mm) alignment.

Treatment Methods

Skeletal transverse deficiencies are often characterized by a narrow palatal vault and posterior dental crossbites. Dental transverse deficiencies are manifested as crossbites or tooth size to arch length discrepancies (crowding). Various skeletal and dental treatment options have been developed in orthodontics to address transverse deficiencies. McNamara divided methods of expansion into three categories: passive expansion, orthopedic expansion, and orthodontic expansion.[8]

Passive expansion occurs when cheek and lip pressure on the dentition are reduced, allowing forces produced by the tongue to move teeth laterally and resolve crowding. Examples of appliances that produce passive dentoalveolar changes include the Frankel apliance and the lip bumper.[3] A study performed by Brieden et al. evaluated the effects of treatment with the FR-2 appliance of Frankel.[9] Using implants for analysis, bone deposition was observed along the lateral aspect of the alveolus.

Orthopedic expansion pertains to increases in the transverse dimension produced by changes in the underlying skeletal structures rather than by dentoalveolar movement. The best example of orthopedic expansion is expansion of the midpalatal suture using a rapid palatal expander (RPE). New bone is deposited in the area of the suture after the palate has been widened. Though RPE treatment aims to produce expansion via suturual separation alone, dentoalveolar expansion via tipping also occurs.[10,11] The dentition is tipped buccally within its alveolar housing. In older individuals, palatal expansion using a jackscrew appliance will produce proportionately less sutural expansion and more dentoalveolar expansion, due to increased bony interdigitations in the palatal suture.[12,13]

Orthodontic expansion is produced by conventionnal fixed appliances with brackets and archwires, as well as by various removable expansion and finger spring appliances. This approach can relieve crowding and improve the transverse dimension of the dental arch, but to a much more limited extent due to the confines of the alveolar housing. With orthodontic expansion, there is a tendency for lateral tipping rather than bodily movement.[3] One of the earliest examples of dental expansion was the use of the E-arch by Edward Angle in the late 1800s. Bands were placed on the molars and were connected by a heavy labial wire that extended around the arch. The archwire could be advanced and elongated to increase the arch perimeter. Individual teeth were ligated to the expansion arch in order to be moved buccally.[12] Fixed orthodontic appliances have continued to evolve, with the latest and most commonly used being the contemporary edgewise appliance.[12] A study by Vajaria et al. evaluated the transverse effects of archwire expansion. Twenty-seven patients were treated using the Damon system. The maxillary 1st premolar width increased by 2.87 mm, and the maxillary 1st molar width increased by 2.79 mm.

The effectiveness of expansion treatments can be assessed by their gains in arch perimeter. Several studies have related expansion to gains in arch perimeter. Adkins et al. evaluated 21 consecutively treated orthodontic patients who received maxillary expansion with a Hyrax appliance. Measurements were performed on pre- and post-treatment dental casts. After a 3-month post-expansion stabilization period, the premolar width had increased 6.1 mm, the molar width increased 6.5 mm, and the arch perimeter

increased 4.7 mm. They found that the maxillary arch perimeter increased 0.7 mm for every 1 mm increase in 1st premolar width.[14]

Germane et al. quantified the amount of arch perimeter gained as a result of dental expansion.[15] Using a mathematical model, they showed that significant amounts of posterior expansion are necessary to gain small increases in arch perimeter, while canine expansion and incisor proclination produce proportionately greater amounts of space.

Table 1: Perimeter Gain with Expansion in Mandibular Arch

Amount of expansion/flaring	**Amount of increased arch perimeter**
1st molar expansion of 1 mm	0.27 mm
1st molar expansion of 5 mm	1.72 mm
Canine expansion of 1 mm	0.73 mm
Canine expansion of 5 mm	5.34 mm
Incisor flaring of 1 mm	1.04 mm
Incisor flaring of 5 mm	6.03 mm

A subsequent study by Motoyoshi et al. repeated similar results for mandibular molar expansion. Using a finite element model analysis, they found that 1 mm of mandibular first molar expansion increases the arch perimeter by 0.37 mm.[16]

In summary, modern orthodontics provides a variety of treatment modalities for addressing skeletal transverse deficiencies and tooth size- arch length deficiencies. Approaches fall into three categories: passive, orthopedic, and orthodontic expansion. To

understand how these treatment methods move teeth, it is necessary to understand the anatomy and biology related to tooth movement.

Histology, Modeling, and Remodeling of Bone

Bone can be categorized as either woven or lamellar depending on its maturational stage. Woven bone is weak and poorly mineralized.[3,17] It is the first bone formed during wound healing and as a response to orthodontic loading. It is remodeled to lamellar bone, which is a strong, well-mineralized bone. After the initial mineralization process, lamellar bone undergoes a secondary mineralization that requires months to complete. The full strength of bone formed after orthodontic tooth movement requires about 1 year.[3]

Typically, mature bones in the body consist of a dense outer sheet of compact bone and a central, medullary cavity consisting of trabecular bone. Both compact and trabecular bone are similar in that they are comprised of microscopic layers called lamellae. The osteon is the basic organizational unit of bone, consisting of cylindrically arranged layers of bone around a central canal called a haversian canal. Capillaries run through haversian canals. Adjacent haversian canals are connected by Volkmann canals, which also contain capillaries. Spaces between osteons are filled with interstitial lamellae, which are fragments of previous osteons. The outer aspect of compact bone is identified as circumferential lamellae because it surrounds the entire bone structure.

Bones grow, adapt, and turn over by two different mechanisms: modeling and remodeling. Bone modeling produces changes in shape or size of bone due to

independent sites of bone formation and resorption. It produces facial growth and is the targeted biologic system for headgears, palatal expanders, and functional appliances.[3] Modeling can be observed by changes in tracings of serial cephalograms. Remodeling, however, can only be observed at the tissue level.

Remodeling describes the internal turnover of mineralized bone without a change in the overall form.[18] It consists of coupled catabolic and anabolic events to control bone metabolism and repair and replace aged or damaged bone. Remodeling is involved in functional loading and tooth movement. The bone multicellular unit (BMU) is responsible for the remodeling of periosteal, endosteal, and trabecular surfaces, in addition to within cortical bone.[19] Today's concept of bone remodeling can be largely attributed to Frost, who developed the use of tetracycline labels to observe turnover events.[20]

The BMU remodeling sequence, also known as the "A-R-F" sequence always begins with activation (A), followed by resorption (R), and then formation (F).[19] This process can be further divided into six phases: Activation, Resorption, Reversal, Formation, Mineralization, and Quiescence. *Activation* describes the recruitment of the osteoclast precursor cells, which requires about 3 days. Osteoclasts are large, multinucleated cells that resorb bone. They originate from hematopoietic stem cells in the monocyte/macrophage lineage and can cytochemically be distinguished from other cells within this lineage by the presence of tartrate-resistant acid phosphatase (TRAP) in cytoplasmic vesicles. Osteonal BMU's can originate on cortical or trabecular bone surfaces. During the *resorption* phase, newly formed osteoclasts begin to resorb bone

along a cutting cone at a rate of about 40 μm per day. The cutting cone is typically oriented longitudinally through the bone. The transition from catabolic to anabolic activity, or the *reversal* phase, takes several days and appears as a cylindrical space between the resorptive region and refilling region. The *formation* phase is marked by osteoblasts laying down bone. Osteoblasts differentiate from mesenchymal stem cells and are recruited to the periphery of the tunnel formed by the osteoclasts. They lay down concentric lamellae at an average radial closure rate of 1-2 μm per day.[19] Osteoblasts do not completely fill in the tunnel so that a vascular supply can be maintained to nourish the osteoclasts and osteoblasts. Osteoblasts deposit unmineralized organic matrix called osteoid. Some osteoblasts become trapped in the matrix they secrete. During the *mineralization* phase, calcium and phosphate minerals are deposited between the collagen fibers. There is a mineralization lag time of about 10 days. About 60% of the mineralization of osteoid occurs within the first few days. This is called primary mineralization. The remaining 40% is added over a 6-month period of secondary mineralization.[21] The quiescence phase begins after the tunneling and refilling processes have completed. The osteoblasts will either remain as osteocytes in the central passageway called the Haversian canal or disappear. The Haversian canal provides a vascular supply to sustain the bone matrix osteocytes and to carry calcium and phosphate to and form the bone as necessary for homeostasis.

Bone modeling and remodeling are both involved in the biomechanical response to tooth movement. Bone modeling involves independent actions of osteoblasts and osteoclasts, whereas remodeling involves coupled activities by the two cell types.[19]

Modeling is substantially reduced after growth is completed. Remodeling occurs continuously throughout life, though it is also reduced after skeletal maturity

Dentoalveolar Anatomy

In order to understand orthodontic tooth movement, it is important to describe the anatomy of the supporting structures, called the periodontium. The periodontium consists of gingiva, alveolar bone, the periodontal ligament, and cementum. The tooth root and its outer cemental layer are held in the alveolar socket by the periodontal ligament. The cementum, similar to the alveolar bone, is a mineralized tissue.[22] An acellular primary cementum is formed during root formation with the main role of providing attachment for the tooth. As a response to functional demands after tooth eruption, cellular secondary cementum is formed and is associated with repair of the PDL in response to tooth wear and movement.[23] Neither primary nor secondary cementum has blood vessels or innervation. Secondary cementum continues to be deposited throughout life.

The PDL is approximately 0.25 mm wide. It is a vascular cellular connective tissue structure that surrounds the root. Fibroblasts are responsible for producing its fibrous extracellular matrix, which consists primarily of collagen. The PDL connects the cementum to the alveolar bone of the surrounding socket via collagen fiber bundles called principal fibers that form as the tooth erupts. They become more numerous and thicker with time. Sharpey's fibers are the terminal ends of the principal fibers that are embedded in the cementum and the bone lining the alveolar socket.[23]

The alveolar process consists of a cortical plate that lines the buccal and lingual surfaces, a central region of trabecular bone, and an alveolar bone that lines the socket. The alveolar bone and cortical plate meet at the alveolar crest.[23] The alveolar bone contains many foramina for nerves and vessels and is sometimes referred to as the cribriform plate. Radiographically, it has increased radiopacity and is referred to as the lamina dura. The term bundle bone is also used to describe this bone that directly lines the socket. It contains the Sharpey's fibers described above. Bundle bone is a functional adaptation of lamellar structure that contains less intrinsic collagen fibers than lamellar bone and upon examination has a course-fibered appearance. Lamellar bone typically covers the bundle bone, but in some areas only bundle bone is found outlining the alveolar socket.[23] Lamellar bone can be either compact or trabecular.[3]

The cortical plate in some regions consists of dense, compact lamellar bone containing haversian systems. It is also referred to as either the buccal or lingual cortex. Interestingly, all histological forms of bone can be found in the alveolar process due to the constant adaptive process resulting from minor tooth movements. The central part of the alveolar process consists of trabecular bone and surrounding spaces occupied by mostly yellow marrow and some red marrow. Large trabeculae consist of lamellae with haversian systems. Trabecular bone is not commonly found in the anterior teeth region, where the cortical plate and cribriform plate are directly adjacent to one another.[23]

The cortical bone is covered by a periosteum, which is differentiated from the surrounding connective tissue. The outer layer of the periosteum is termed the fibrous layer and contains dense, irregular connective tissue. The inner layer consists of bone

cells and their precursors, as well as a microvascular supply. The periosteum functions as an osteogenic zone throughout life due to the presence of osteoblasts and their progenitors.

Biology of Tooth Movement

Orthodontic tooth movement occurs when an external force disrupts the physiologic equilibrium of the tooth within its surrounding structures, encouraging its movement until a new point of equilibrium is achieved.[12] Krishnan and Davidovitch showed that mechanical forces applied to the teeth are transferred through the PDL to the alveolar bone, which produces a cellular response.[24] The mechanical strains alter the PDL's blood flow, resulting in release of neurotransmitters, cytokines, and other molecules that evoke responses from different cell types to stimulate deposition or resorption of bone.

Several theories have been developed to describe tooth movement, of which the first was the Pressure-Tension Theory. Research by Sandstedt in 1905 demonstrated that tooth movement is a process of resorption and apposition. Using a dog model, he moved maxillary incisors lingually and noted that bone was deposited on the tension side and resorbed on the pressure side. He observed that heavy forces could occlude capillaries and result in cell death. He was the first to describe this compressed, cell-free tissue as hyalinized.[25] In 1932, Schwarz found that if the capillary bed blood pressure is exceeded (20-25 gm/cm of root surface), tissue necrosis results.[26] The Pessure-Tension Theory was based on histologic evaluation of the periodontium. It was observed that width

changes in the PDL cause disruption in collagen fibers on the compression side. Hyalinized tissue contains pyknotic nuclei and areas of cell-free zones. In hyalinized regions of the PDL, cells cannot differentiate into osteoclasts to resorb bone. Thus, hyalinization slows down tooth movement. The tooth will not move until the underlying bone, along with the necrotic tissue, has been resorbed. Osteoclasts and macrophages from adjacent undamaged medullary spaces must remove this necrotic tissue as well as the bone surface through a process called undermining resorption.[24,27,28] A hyalinized area formed by application of light forces will persist for 2-4 weeks in young patients.[3] Once the hyalinized zone and adjacent bone surface has been resorbed, the PDL is reestablished in a wider ligament space, and this new membrane is rich in cells that produce new periodontal fibers.[2,3]

The Pressure-Tension Theory holds that inflammation caused by orthodontic tooth movement can induce cell recruitment and tissue remodeling.[24] When light, favorable forces are applied, osteoclasts will appear along the bone surface of the pressure side and resorb bone directly. This form of resorption, termed frontal resorption, allows for faster tooth movement as compared to undermining resorption. On the tension side, new bone is deposited once osteoblasts have proliferated. Bone is laid down until the width of the membrane returns to its normal size. Remodelling of the PDL fibers also occurs to accommodate the new tooth position.[23]

Reitan further studied the histologic changes and found that the amount of degeneration was related to the amount of force per unit area.[2,29] He noted that hyalinization in the PDL resulted from tipping movements even with minimal force.

This would lead to undermining resorption at the crestal region. The amount of hyalinization was also greater in the case of short roots. However, very little hyalinization was observed during translation, or bodily tooth movement. Translation also favored direct bone resorption rather than undermining resorption.

In 1969, Baumrind proposed an alternative theory called the Bone-Bending Theory. He argued against the orthodontic popular belief at the time that tooth movement occurs only through changes within the PDL.[30] He stated that orthodontic forces are transmitted to all tissues in the region of force application: tooth, PDL, and bone. This results in their deformation based on each structure's elastic properties. The bone deforms far more than the PDL because the PDL appears to have a relatively stable dimension. His hypotheses were based on the findings of his study in which dimensional changes between the maxillary first and second molars in 99 rats were studied after placement of elastic bands. The study showed bone deflection occurring with forces lower than those required to produce changes in the PDL width. He explained that although fibers are present, the PDL is a body of liquefied ground substance that follows Pascal's Law. Forces delivered to the PDL would be transmitted throughout itself since it is a continuous hydrostatic system.[30] Because the PDL is highly viscous and rubbery, he argued that it does not make sense for the tissue fluids to be "squeezed out" from the PDL to allow the tooth to move within the PDL space. Baumrind's work showed that bone bends in response to force application. Studies by Muhlemann and Zander in monkeys, as well as Grimm in humans, supported Baumrind's findings by showing dentoalveolar displacement when forces were applied to the teeth.[31,32] This bending

activates biologic responses that involve bone turnover and renewal.[30] Bone turnover is accelerated while the bone is held in its new position. This results in stress line formation in the deflected bone, which in turn stimulates biologic activity to modify the shape so that it accommodates the new forces placed on it. Reorganization occurs in the lamina dura as well as on the surfaces of all the trabeculae within that region of bone.

To further understand this theory on orthodontic bone deflection, orthopedic concepts related to mechanical adaptability of curved bones must be introduced. Physicians have noted for many years that fractured long bones healed in a bent configuration will straighten themselves over time. Jansen, among others, explained that if a bent bone is to be straightened, bone must be added on the concave side and removed on the convex side.[33] Load placed in an axial direction will produce tensile stresses on the convex side and compressive stresses on the concave side. Tension stimulates resorption and compression stimulates formation, so that eventually the curved long bone will appear straighter. Frost furthered this concept by noting that the medullary canal also exhibits a modeling response to loading. If a periosteal surface receives compression, the contiguous endosteal surface will experience tension, and vice versa.[34] Furthermore, he theorized that the applied end load has a tendency to alter the relative curvature of the bone surface. He suggested the association that increased surface convexity led to bone resorption, and decreased surface convexity led to bone formation. The strain level required had to be above a minimum threshold. Otherwise, a bone's preexisting curvature would be controlled to keep strain levels within a physiologic range.[19]

In applying these principles to the periodontium, research performed in dog mandibles demonstrated that canine tipping bends the alveolar bone, creating a convex surface on the "pressure side" and a concave surface on the "tension side."[35,36] Epker and Frost showed that stretching of the PDL fibers causes bone bending in this tension zone and results in apposition of bone.[37] The orthopedic description would be that the bone near the stretched PDL fibers is actually under compression rather than tension, because it has become concave. This concave region of loading would induce the laying down of bone. Similarly, whereas orthodontists relate loading to resorption, orthopedists would say the loaded area assumes a convex shape and is under tension, thus inducing resorption.[19]

Frost formulated another theory to relate mechanical loading to biological reactions, called the "Mechanostat Theory." This theory sought to correlate resorption and formation to levels of bone strain.[38] If strain values are below a "minimum effective strain," a net loss of bone occurs due to increased modeling. As strain increases above the minimum effective strain, modeling is initiated as an adaptive response and a positive balance occurs. This is an adaptive process that allows bone to become more stress resistant. When the strain is at a neutral amount, resorption and apposition occur, and the new bone is lamellar. When larger strains are reached, woven bone is formed instead. Even higher strains result in more bone being resorbed than added, since repair cannot keep up with the amount of microfractures. Beyond a certain amount, the fracture strength is reached and pathologic fracture occurs.

Melsen sought to test Frost's Mechanostat Theory in relation to the biological

reaction of the periodontium to applied levels of strain.[39] Six *Macaca fascicularis* monkeys were used in the study. The first and second molars were extracted so that the second premolar and third molar could be translated for 11 weeks. Interestingly, they found that the strain values in the direction of tooth displacement were below the minimum effective strain. According to the Mechanostat Theory, this would stimulate remodeling with a net loss in bone mass, which is in fact what occurred. Furthermore, the stretched PDL fibers on the opposite side generated a strain level corresponding to modeling in Frost's theory, and new bone formation was observed.

The Bioelectric Theory was another theory used to explain tooth movement. It is not mutually exclusive of the theories mentioned thus far. In 1962, Basset and Becker proposed that electrical potentials are generated in stressed tissues in response to mechanical forces.[36] Several experiments have shown that stress-generated electrical potentials are associated with modeling and remodeling. In 1970, Friedenberg et al. showed that bone formation is stimulated near a cathode and resorption is stimulated near an anode.[40] This was supported in 1974 when Zengo et al. measured the electric potentials in mechanically stressed alveolar bone in dogs and found that the concave side of orthodontically treated bone is electronegative and favors osteoblastic activity, whereas the convex side is positive and showed elevated osteoclastic activity.[35] Further research by Davidovitch et al. showed that bioelectric responses produced by bone bending after orthodontic force application can act as cellular first messengers to enhance the remodeling response.[41,42] They further found that mechanical movements of about 1 minute per day can cause an osteogenic response.

Optimal Force Levels

The force magnitude, as well as the direction and duration are all important components to tissue reactions and the resulting tooth movements. The typical schematic for tooth movement has three phases: initial strain, lag phase, and progressive tooth movement. After application of a continuous, moderate load of about 20 to 50 grams, initial strain occurs during the first week with 0.4 to 0.9 mm of movement due to PDL and bone displacement. The lag phase describes the slow period of tooth movement when undermining resorption must occur to remove hyalinized tissues before the tooth can become further displaced. This phase can last form 4 to 20 days.[24] The third stage of progressive tooth movement describes the period in which frontal resorption limits the rate of tooth movement.[3] This concept is a general and simplistic view of the complex fluid and solid mechanics involved in tooth movement.

Quinn and Yoshikawa studied the relationship between the magnitude of applied force and the amount of tissue reaction.[43] They developed four alternative hypotheses for this relationship. The first described a threshold force level that must be reached in order for tooth movement to occur, and once met, the rate of tooth movement is the same regardless of the force level. The second hypothesis suggested a linear dose response relationship with a threshold force level. The third hypothesis described that a dose-response relationship existed in the lower force range, but only up to a certain level. Further increases in force level beyond this point would decrease the rate of tooth movement. In the fourth hypothesis, the dose response relationship existed to a certain level, after which there was no further increase or decrease in tooth movement rates with

increases in the amount of force. They found that previous studies on stress levels and rates of tooth movement supported the fourth model, in which increasing the force did not increase the rate of movement after the maximum rate was achieved. Based on clinical data, their estimate for a maximally efficient canine retraction force was 100 to 200 gm. This would yield a mean compressive stress on the canine root of approximately 70 to 140 gm/cm^2. The conclusion was that efficient tooth movement, particularly of canine retraction, involved the continuous application of a maximally efficient force level. Their observations led to the conclusion that force levels play a lesser role in orthodontic tooth movement.

A study by Boester and Johnston compared four different force levels for canine retraction in 10 adolescent patients: 2 ounces (55 gms), 5 ounces (140 gms), 8 ounces (225 gms), and 11 ounces (310 gms).[44] The 2 ounce force resulted in significantly less movement than the 5, 8, and 11 ounce forces, but these three greater force levels produced about the same amount of retraction. Data by Pilon et al. in dogs further supported this finding.[45] No difference in rate of premolar movement was observed between forces of 50 and 100 cN (~50 - 100 gms). A study by Owman-Moll et al. found that increasing the force level did not increase the rate of tooth movement after the application of at least 50 cN.[46]

In 2003, Ren et al. performed a systematic review of the existing literature to determine the optimal force level in orthodontics.[47] They concluded that no evidence-based force level could be recommended for optimal efficiency. This article was followed up with the development of a mathematical model. They used experimental

data form four dog studies to produce a mathematic equation predicting a maximum average rate (with a 95% CI) of 0.23 to 0.30 mm/week. The forces used in the model ranged from 10-1200 cN, and the optimum force magnitude was found to range from 104-454 cN. Their findings suggested that the force level is not a major decisive factor for the rate of tooth movement.[48] von Bohl et al. argued that it is not possible to describe the rate of tooth movement based solely on the amount of force. The degree of PDL hyalinization, the rate of removal of necrotic tissue, bone morphology and density are important factors in determining the response to orthodontic forces.[49,50]

Force Systems and Biologic Responses

It is important to note that the biologic effect of force application is not determined by the force level alone, but by the force per unit area.[51] The distribution of force within the PDL will differ based on the type of orthodontic tooth movement. Tipping is the simplest tooth movement to produce. When a force is applied to a tooth at a single point, the tooth will rotate around its center of resistance. The center of resistance is the point at which resistance to tooth movement can be concentrated for mathematical analysis.[12] Since the root is embedded in bone, the center of resistance must be approximated. It is located about halfway between the root apex and crest of the alveolar bone in a single rooted tooth. When a tooth tips, the PDL is compressed at the crest of the alveolar bone in the direction of tooth movement, as well as at the root apex on the opposite side. The highest level of pressure is concentrated at the crest and apex, and the pressure dissipates towards the center of resistance. It is typical for hyalinization

to occur below the alveolar crest.[3] Since tipping results in high-pressure zones, the force level must be kept low to decrease hyalinization and crestal resorption. For a single rooted tooth, the force level should not exceed 50 gm when tipping.[12]

A torqueing movement describes tipping of the apex. The pressure area is located closer to the apex, and if excessive torque is applied, fenestration of the buccal bone plate can occur. Translational movement, also described as bodily movement, requires that two forces be applied simultaneously to the crown of a tooth in a specific balance to allow for both the tooth and root to move the same amount. The entire PDL surface is loaded in the direction of tooth movement. About twice as much force is required for bodily movement as for tipping. Other tooth movements include rotation along its long axis, extrusion, and intrusion, each of which requires much less force compared to translation (35-60 gm).

Mesio-Distal versus Lateral Tooth Movement

The biology and mechanics of mesio-distal tooth movement differ significantly from lateral tooth movement. During mesio-distal tooth movement, the tooth must first travel through the compact bone of the cribriform plate.[51] Once the cribriform plate is resorbed, the tooth will move through primarily trabecular bone in addition to a smaller amount of cortical bone located at the alveolar crest. When a tooth is moved laterally, it must travel through the cribriform plate and variable amounts of trabecular bone, if any, before reaching the dense buccal cortex. Thus, lateral tooth movement is often slower and more difficult. If the alveolar process into which the tooth is being moved is

extensively resorbed, such as an edentulous site, the tooth may need to move through a greater amount of cortical bone. If the tooth is being moved through a recent extraction site, it will more easily move through this trabecular bone, some of which may still be immature woven bone[3].

Mechanically, it is also more difficult to control lateral tooth movement compared to mesio-distal movement. During both forms of tooth movement, the location of force application is typically above the center of resistance (i.e. at a bracket on the buccal surface). Because there is a distance between the center of resistance and the location of force application, a rotational movement called a moment is created. The tooth will tip as it is moved in the direction of the force. A separate force created when the wire engages the entire width of the bracket slot while tipping counteracts this rotational movement. This separate force is called a couple. The couple provides the moment necessary to counteract the moment of the force applied to the crown of the tooth in the mesio-distal direction. When a tooth is moved laterally, the bracket depth rather than the bracket width creates this couple. The depth is about ¼ the width (0.028 in. versus 0.1 in.) of the bracket, which makes controlling bucco-lingual movement much more difficult than mesio-distal movement. The opposite edges of a rectangular wire would serve as the two points of contact to produce a torqueing couple, but because the moment arms of this couple are quite small, the force at the bracket necessary to create this counterbalancing moment is very large. Thus, it is more difficult to achieve bucco-lingual translation as compared to mesio-distal translation.

In order to decrease the magnitude of the moment that causes a tooth to tip buccally, the force must be applied closer to the center of resistance. This can be achieved by constructing a rigid attachment projected over the center of resistance of the tooth. If the buccal force is applied at this attachment, the moment arm will be reduced or eliminated and the tooth will be allowed to translate.[12]

Effects of Arch Development on Buccal Bone

In 2011, Cattaneo et al. studied transverse tooth movement and buccal bone modeling using active (In-Ovation R) and passive (Damon) self-ligating bracket systems.[52] CBCT scans were taken before and after treatment of 64 patients. Transverse expansion of the maxillary arch by buccal tipping occurred in all but one patient in each group. The Damon group showed 11.7 degrees of tipping at the upper first premolars and 13.5 degrees of tipping at the upper second premolars. The In-Ovation R group showed 11.8 degrees and 13 degrees of tipping, respectively. CBCT measurements showed a 20% decrease of buccal bone area in the Damon group and a 14% decrease in the In-Ovation group. It was concluded buccal bone modeling did not accommodate the buccal tooth movement, resulting in decreased alveolar bone support.

Kraus et al. experimentally demonstrated the negative consequences of buccal tipping on crestal bone.[4] The 2nd premolars in 7 male beagle dogs were expanded with archwires using a passive self-ligating system, which applied between 73-178 gms of buccal force. Results showed that the 2nd premolars tipped buccally about 15.8 degrees and expanded 3.5 mm when measured at the incisal edge. Expansion produced

dehiscences of 2.9 mm at the mesial root and 1.2 mm at the distal root.[4] Ruso et al. similarly showed dehiscences resulting from archwire expansion and tipping of teeth.[53]

Studies on Buccal Plate Encroachment During Tooth Movement

In 1973, Wainwright studied the effects of moving root apices through the cortical plate.[54] He used four *Macaque speciosa* monkeys; four procedures were performed in each monkey (one per quadrant). In the first quadrant, the second premolar's root apex was tipped through the buccal plate and into the soft tissue with no retention after movement. It took 4 months for the apex to be located into the soft tissue. In the second quadrant, the root was moved through the buccal plate and then retained for 4 months. In the third quadrant, the root apex was moved through the buccal plate for 4 months and then actively moved back into the alveolus for 3 months. In the fourth quadrant, the root was moved out of the buccal plate, then moved back, and then retained for 3 months. He applied force levels that he considered to be optimal for root movement in human beings. The force level was kept constant to maintain a constant axis of rotation. The forces used were 500 gm-mm and 760 gm-mm for the maxillary and mandibular second premolars, respectively.

Histology of the first quadrant showed that the root apex had no overlying buccal bone and was in the buccal soft tissue. Interestingly, the buccal plate was undergoing remodeling, with many osteoblasts aligned on the periosteum. Bone deposition had occurred on the buccal surface of the cortical plate around the perforation, while the lingual side of the cortical plate displayed osteoclast activity. Root resorption occurred

on the buccal surface, with increasing severity toward the apex. The PDL fibers had lost their normal arrangement and took on various orientations. At the location of fenestration, the PDL and periosteum merged and were continuous, containing collagen fibers and fibroblasts. Areas of hyalinization were very rare, though root resorption was present on the buccal surface. Histology of the second quadrant demonstrated more bone covering the root apex and the cortical plate in the vicinity of perforation was thicker than in the first quadrant. The PDL appeared less disorganized with a more uniform thickness. The third quadrant surprisingly showed complete restoration of the original contour of the buccal plate. The Procion dye marked the location of the cortical plate penetration of the apex. The first bone to be laid down at the fenestration had remodeled into a denser and more compact bone. The root showed resorption on both buccal and lingual surfaces. The PDL resembled the first quadrant with disorganized fibers, but was slightly more uniform. In the fourth quadrant, the perforation on the buccal cortical plate had completely repaired with slight increase in width due to compensatory bone formation. The buccal plate had remodeled into new compact bone. The PDL was uniform in thickness and there was some return to the normal organized state of fiber orientation. This study showed that if the root was moved back and retained in the original position, complete repair of the fenestration was possible, with even further slight thickening of the cortical plate.

In 1981, Steiner et al. performed a study to assess hard and soft tissue defects related to buccal tooth movement.[1] Five *Macaca nemistrina* monkeys were used to move the central incisors labially a mean of 3.05 mm over 13 weeks with a 50 gm force.

Periodontal flap surgery was performed, revealing significant gingival recession, decreased connective tissue, and loss of buccal bone height. Eight months after this study, Engelking and Zachrisson studied the same set of animals and moved the incisors back into the alveolus using fixed appliances. The incisors were retracted an average distance of 1.8 mm and retained for 5 months. Histological analysis showed that the marginal bone levels recovered 2.5 mm at the maxillary incisors and 3.1 mm at the mandibular incisors. Tetracycline labeling also revealed significant new bone formation around the retracted teeth. They concluded that labial bone regeneration can occur when displaced teeth are moved back to their original positions.[55]

Karring et al. in 1982 performed a similar study with 6 beagle dogs to evaluate facial tipping of the maxillary second and third incisors.[56] Three dogs received orthodontic appliances and the other three served as non-treated controls. The left maxillary incisors were tipped for five months in a facial direction through the alveolar bone plate using orthodontic appliances. During the subsequent 5-month period, the left side incisors were tipped back to their original position, while the right side incisors were moved facially to the position previously reached by the left side incisors. The same orthodontic appliances were used to retain the teeth for 5 months. After 15 months, the animals were sacrificed for histological analysis. No significant loss of clinical attachment was shown. The average distance between the bone crest and CEJ in the controls was 2.2 mm. The average distance between the bone crest and CEJ in the group in which the incisors were moved back after tipping was 1.8 mm, and 4.1 mm in the group in which the incisors were retained in the displaced position. There was no

significant difference in bone heights between the controls and the group in which the incisors were moved back after tipping. This again supports the concept that though dehiscence forms when teeth are tipped, such defects can be repaired if the teeth are moved back.

Noting that bone regenerated after buccally moved teeth were moved back lingually, Thilander postulated that a bone matrix remains in the soft tissue buccal to the bone dehiscence.[57] This bone matrix has the capacity to remineralize when a tooth is returned to its original position in the dental arch. He speculated that genetic factors control the dimensions of the alveolar process and do not allow bone to form outside the confines of the alveolar process. While these studies support repair of iatrogenic dehiscenses when teeth are moved back to their original position, the literature also supports bone modeling in response to the movement of teeth to a new bucco-lingual position.

Alveolar Bone Modeling in Response to Lateral Tooth Movement

In 1976, Edwards performed a study of the anterior palate.[58] Cephalograms of 188 patients were obtained. ANB angles ranged from 6 to 11.5 degrees. Following the removal of the first premolars, the anterior teeth were retracted using maximum anchorage. Six of the patients received metallic implants to measure the labiolingual dimension of the palate during retraction. Three were placed in the midline of the palate behind the upper incisors, and two were placed on the labial cortex. The cases were divided into two groups depending on the original width of the anterior maxilla. Group 1

displayed adequate labiolingual width of the alveolar process to accommodate incisor retraction. Group 2 had labiolingual alveolar widths that were too narrow to accommodate the necessary incisor retraction. In Group 1, bone lingual to the incisors did not change in width or A-P position even though the incisors were moved lingually. Also, the alveolar bone widths labial to and lingual to the incisors did not change (i.e. maintained the same thicknesses as before treatment). This is contrary to the expectation that new bone would have been laid down on the palatal side to keep the incisor root within the middle of the alveolar process. Measurements showed that as the incisors were moved lingually, the labial plate moved lingually as well, but to a lesser extent. Because no new bone was added to the palatal surface, resorption of the labial bone resulted in a thinner alveolar process in this group.

In Group 2, the alveolar process was modeled as it assumed a more vertical orientation in both adults and growing patients. The amount of change was smaller near the root apex and larger near the bone crest. In the marginal and midroot regions of alveolar bone, the palatal bone moved lingually along with the labial bone as the incisors were retracted.

Of the six patients in whom metallic implants were placed, only two were technically acceptable. They showed that the palatal cortical plate moving lingually by osseous apposition because the implants were shown to be “left behind” and surrounded by cancellous rather than cortical bone. The implants on the labial shifted position under the periosteum due to resorption of the cortical plate. The most apically positioned implant, however, did not change position since this portion of the buccal plate did not

change appreciably during retraction. The implant study refuted the theory of "bending" of the alveolus as a means of altering the position of the alveolar bone. Rather, if tooth movement was required beyond the initially existing alveolar process, a combined apposition and resorption process occurred. If either the A-P position of the alveolus or the width was insufficient for the retraction necessary, bone modeling occurred.

To examine transverse dentoalveolar changes, Corbridge et al. (2011) studied the effects of quad-helix expansion on the bone surrounding the maxillary first molars.[59] CBCT scans were obtained before phase 1 (mean age, 9.2 years) and phase 2 (mean age, 11.9 years) treatments in 73 consecutively treated patients. The size 3 quad helix was used in most patients and produced approximately 441 gms of force. Slow palatal expansion decreased buccal bone thickness (1.6 mm ± 0.8 mm), and increased lingual bone thickness (1.6 mm ± 1.3 mm) and alveolar width (0.5 mm ± 1.0 mm). At the beginning of phase 2 treatment, one third of the patients showed little or no buccal cortical bone on at least one side. Intermolar widths and palatal widths also increased. They concluded that the teeth moved through and with the alveolus, causing decreases in buccal bone thickness and increases in lingual bone thickness. The alveolar bone appeared to adapt to the increased intermolar width by apposition of small amounts of new bone on the buccal surface.

As previously described, Kraus et al. achieved buccal movement of the maxillary second premolars in beagle dogs at the expense of crestal bone.[4] After moving the teeth buccally for a period of 9 weeks, significant loss of bone height occurred, in addition to significant thinning of the bone, as measured using microCT. Buccal tipping resulted in

bone apposition on the trailing edges of tooth movement (cervico-palatal and apico-buccal regions). This was demonstrated by red and green fluorescent labeling of new bone in histomorphometric sections. H&E sections also demonstrated increased cellular activity, primarily with greater numbers of osteoblasts. Interestingly, bone apposition was also evident on the periosteal surface of the crestal portion of the buccal plate. This indicated that new bone was laid down ahead of the teeth being moved. Histomorphometric sections showed a change in the pattern of bone apposition, with buccal bone being added on the periosteal side near the crest but on the PDL side more apically. They explained that this reversal zone demonstrated the location of the center of rotation of buccal tipping. New bone was also evident on the leading edge of the apex near the maxillary sinus.

In a subsequent study, Capps applied translatory forces to maxillary second premolars in orthodontic patients to study the effects on the surrounding hard tissue.[60] His study differed from previous studies in that a cantilever arm was used to produce buccal translation with minimal crown tipping. A 50-gram buccal force was applied for approximately 9 weeks. Pre and post treatment CBCT's were analyzed and superimposed to evaluate changes in the dentoalveolar complex. The results demonstrated 0.96 mm of buccal tooth movement with minimal tipping (2.2°). Buccal bone heights decreased by a minimum of 0.25 mm and a maximum of 0.60 mm. Buccal bone thickness 3mm apical to the CEJ decreased by 0.63 mm. However, based on CBCT measurements, buccal bone increased in thickness by 0.46 mm and 0.51 mm,

respectively. He concluded that light, continuous forces with limited amounts of tipping could produce limited amounts of new bone.

Conclusions

Buccal expansion is a common treatment for transverse deficiencies and crowding. Current methods of lateral tooth movement cause significant tipping and crestal bone loss. If tipping is mitigated, recent research shows that light, continuous forces can produce small amounts of new bone. Histologic analysis is necessary to definitively show whether new bone is added on the periosteal surface of the buccal plate. The present study utilizes the dog model to study the effects of buccal translation. Histologic analysis includes microCT, fluorescent imaging, and histologic stains to assess new bone formation and turnover. The information gathered in this study provides insignt on the effects of orthodontic expansion on the buccal bone in the absence of tipping.

CHAPTER II

EFFECTS OF TRANSVERSE, BODILY MOVEMENT OF MAXILLARY PREMOLARS ON THE SURROUNDING HARD TISSUE

INTRODUCTION

Transverse maxillary deficiencies and crowding are common problems among orthodontic patients. Clinical approaches to correct such problems include the use of rapid maxillary expansion (RME) and archwire-assisted expansion. RME corrects transverse arch deficiencies by skeletal separation of the palatal suture. Dental expansion with archwires uses light forces to increase the transverse dimension, primarily with tooth movements.

A well-documented negative effect of dentoalveolar expansion, performed using any current non-surgical approach, is dental tipping and subsequent loss of buccal alveolar bone.[1-3,56] Expansion causes uncontrolled tipping, or tipping of the crown buccally relative to the root, which concentrates pressure at the most coronal portion of buccal alveolar bone, leading to resorption of the crestal bone.[2,3] It is thought that bone loss occurs due to microfractures that occur when the bone is strained beyond its adaptive capabilities.[38] The damaged bone cannot be remodeled quickly enough and is consequently resorbed, producing dehiscences.

Uncontrolled tipping occurs because the forces during dentoalveolar expansion are applied occlusal to the tooth's center of resistance. To avoid dental tipping, it is necessary to utilize an appliance system that delivers a buccal force near the center of resistance of the tooth, or an equivalent force system. Such a force application results in

bodily, transverse movement, which distributes the forces over a larger surface area along the root, resulting in less crestal bone loss.[2] By distributing forces, strains could be reduced to be within the physiologic range necessary to produce active bone modeling and increase bone mass.[38,39,61-63] Within the physiologic range, bone loss and growth are adaptive processes that allow bone to modify its mass and geometry based on functional needs.[38]

A recent experimental study evaluating orthodontic expansion showed new bone formation on the periosteal surface of buccal cortical bone after orthodontic expansion.[4] If excessive tipping could be avoided, would dehiscences be eliminated and would there be bone growth along the entire periosteal surface? Or worded differently, when teeth are moved buccally, is it possible to produce the amount of strain required to model bone and to increase bone mass on the buccal plate? Recently, Capps et al. found that bone mass could be added on the buccal plate as a result of controlled buccal translation using a cantilever that pulled from the center of resistance of the maxillary premolar.[60] Radiographic assessments showed that the experimental teeth moved buccally an average of 1 mm with very limited tipping, adding approximately 0.5 mm of bone to the buccal plate. Unless histology is performed, however, it remains impossible to determine whether this was new bone. Since bone is able to deform within its own elastic limits,[30,31] it is possible that the cortex could have been displaced buccally during translation.

Our understanding of buccal alveolar bone adaptation to controlled tooth movement is extremely limited. The goal of the present study was to evaluate the effects of buccal translation on the surrounding bone. By furthering our understanding of bony adaptation to buccal expansion, orthodontists will be better able to make treatment decisions to maintain the health of the periodontium and surrounding dentoalveolar bone.

MATERIALS AND METHODS

Seven skeletally mature, periodontally healthy, beagle dogs between 1 to 2 years of age and weighing 21-29 lbs. were used in this study. Dogs were used because their bone composition and turnover rates are similar to those of humans.[64-66] Housing, care, and the experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC #2014-0275-BCD) at Texas A&M University Health Science Center, Baylor College of Dentistry. The animals were maintained in good health with proper diet and nutrition. They had fully erupted dentitions with no missing teeth.

Initial Records

Using an electronically generated random number table, one side of the maxillary arch was randomly assigned to receive an active buccal expansion appliance ("experimental side"). The other side received no appliance ("control side"). Following 10 days of quarantine, initial records were obtained. The animals were sedated with an intramuscular injection of Ketamine (8-24 mg/kg IM) mixed with Xylazine (0.22 mg/kg IM). Ultrasonic prophylaxis and mouth rinse with 0.2% chlorhexidine gluconate were performed. Initial records consisted of intraoral photographs, periapical radiographs, and alginate impressions. Periapical radiographs were obtained on the experimental sides using a size 4 phosphor plate (Air Techniques Inc., Melville, NY). They were acquired using a custom holder that stabilized the film and x-ray tube at reproducible angulations and distances. A custom measurement jig was made using Triad TruTray (Dentsply Intl., York, PA) and two 10 mm-long stainless steel wires oriented in an occlusoapical

direction. The Triad material was molded to fit over the crown and the two vertical wires were positioned to rest over the buccal mucosa on either side of the second premolar roots (Figure 1A). Alginate impressions were taken using custom trays made of Triad material. Models were poured using die stone for the fabrication of occlusal radiographic guides and appliances.

Appliance Design

The appliance incorporated the maxillary canine, 2nd premolar, and 4th premolar on the experimental side. It was designed to produce buccal translation of the 2nd premolar, using the canine and 4th premolar as anchor teeth. Orthodontic band material (Dentaurum, Ispringen, Germany) was custom pinched and welded to fit the maxillary canine, 2nd premolar, and 4th premolar. The inner surfaces of the bands were micro-abraded with 60-micron alumina particles. Several small holes were made with a 0.25 round bur to enhance retention. Tubes with a 0.022" slot size (3M Unitek, Monrovia, CA) were welded to the bands on the canine and 4th premolar. The canine tube had a 0° offset, 0° torque, and a 0.051" headgear tube. The 4th premolar tube had a 10° distal offset, -14° torque, and a 0.045" headgear tube. Tubes were welded and then soldered to the bands. To stabilize the canine and 4th premolar, and to act as protection from the cheek, a 0.045" stainless steel wire was fit passively in the headgear tubes. The ends were covered with solder and polished smooth for animal comfort (Figure 2A).

A stainless steel vertical extension arm 1.5 mm in diameter was welded onto the second premolar band (Figure 2A). A 10 mm 0.045" wire segment, which was soldered

perpendicular to the extension arm at the apical extent, served as a cheek guard. The vertical extension arm was positioned 1 mm distal to the crown tip due to the root anatomy. The distal root is wider and is typically tipped distally, while the mesial root extends vertically (Figure 1B).

The apical extent of the extension arm was positioned at the same level as the estimated center of resistance, which has been reported to range from 24% to 60% of root length.[67-69] Since the furcation was located coronal to the alveolar crest, the 2nd premolar was treated as a single rooted tooth, and the center of resistance was estimated to be located half way between the root apex and alveolar crest. Periapical films were imported into Dolphin Imaging (Dolphin Imaging and Management Solutions, Chatsworth CA) and traced. The center of resistance for each experimental second premolar was calculated as follows (Figure 1B).

1. Two lines were drawn connecting the mesial and distal bone levels to the furcal bone level.
2. Line X was drawn connecting the furthest ends of the two lines.
3. One line was drawn connecting the two apices.
4. Line Y was drawn connecting these two lines to get an estimate of the root length.
5. Line Z was drawn from Line X to the crown at the location of the gingival margin. The gingival margin location was determined by examining the relationship of the soft tissue to the crown intraorally.

6. The midpoint of the root length was calculated by dividing Y by two. This number was added to Z to determine the proper length of the extension arm.

Appliance Delivery

On the day of appliance delivery, the animals were initially sedated with the previously described Ketamine and Xylazine cocktail after a 12-hour fast. Vital signs were monitored throughout the procedure. Small notches were made in the cusp tips of the second premolars using a mosquito shaped micro diamond bur (Brassler USA, Savannah, GA). They served as replicable reference markers for digital caliper measurements. Amalgam markers, approximately 1.5 mm in diameter, were then placed using a 330 carbide bur in the canine, 2nd premolar, and 4th premolar on the experimental side for radiographic measurement of tooth movement. Two 6 mm Imtec miniscrew implants (3M Unitek, Monrovia, CA), placed in the midline of the palate just mesial and distal to the 2nd premolars, served as references for the radiographic measurements. After placement, the miniscrew heads were sectioned using a metal cutting bur down to the level of the palatal tissue so that the tissue could heal and cover the screw. Local anesthetic was administered prior to implant placement via local infiltration of 2% Lidocaine with 1:100,000 epinephrine (Patterson Dental, St. Paul, MN).

Following amalgam marker and miniscrew placement, occlusal radiographs were obtained. The orientations of the images were standardized with custom radiographic guides fabricated for each dog using each maxillary stone model. Channels were cut into a 1.5 mm acrylic sheet to fit precisely over the canine and 4th premolar cusp tips. Triad

material was placed within each hole and molded around the cusp tip for added stability. The phosphor plates were centered on the acrylic guide in a standardized location. A 20 mm stainless steel wire was embedded in the template for image calibration. An XCP external paralleling device (Dentsply-Rinn, Elgin, IL) was used for x-ray tube orientation.

Retention grooves were placed in each of the banded teeth with a 330 carbide bur. The teeth were etched with 37% phosphoric acid for 30 seconds and rinsed for 10 seconds. Bands were cemented with RelyX Unicem resin cement (3M ESPE, St. Paul, MN). Excess cement was removed to prevent gingival irritation.

A 0.021" x 0.025" Beta Titanium wire (3M Unitek, Monrovia, CA) was bent to act as a cantilever delivering a 100-gram buccal force on the second premolars. The cantilever force level was adjusted at a bend at the fourth premolar slot and checked using a Correx Tension Gauge (Haag-Streit, Bern, Switzerland). The wire was first engaged in the second premolar tube and cinched distal to it. The loop at the end of the wire was tied to the tip of the vertical extension with a 0.012" stainless steel ligature tie. Coe-Pak periodontal dressing (GC America, Alsip, IL) was applied to the apical extent of the vertical arm to prevent irritation of the mucosa. The other end of the wire was cinched gingivally distal to the 4th premolar tube. Triad Gel (Densply International, York, PA) was applied to the distal tip to prevent mucosal irritation. The occlusion was checked for interferences.

Pocket depths of the control and experimental second premolars were obtained using a periodontal probe. Measurements of the mesiobuccal, buccal, distobuccal,

mesiolingual, lingual, and distalingual regions were obtained in duplicate and averaged. Occlusal radiographs were again taken to ensure that the amalgam markers were not blocked by the metal bands on the radiograph. Photos were also acquired.

Following appliance delivery, the animals received Nalbuphine (2 mg/kg IM) once daily for two weeks. For prevention of post-operative infection, Clindamycin (11 mg/kg IM) was also administered twice daily for two weeks. The animals were maintained on a soft diet for the duration of the experiment to prevent appliance breakages.

Records, Expansion Protocol, and Fluorescent Bone Labels

Data were collected by one investigator every two weeks, starting on the day of appliance placement. After sedation with the previously described Ketamine and Xylazine cocktail, the Coe-Pak was removed and dental prophylaxis was performed as previously described. Interpremolar widths were measured in triplicate at the cusp notches using a digital caliper (General Tools, New York, NY). Replicate measures produced an intraclass correlation of 0.996. Periodontal probing depths were obtained as previously described. Force levels of the wires were recorded and occlusal radiographs were obtained every two weeks before the wires were replaced. Occlusal radiographs were obtained, and the existing wire was replaced with a new segment of wire and activated to 100 grams, which was verified using the Correx Tension Gauge. Coe-Pak and Triad gel were replaced on either end for comfort, and photos were acquired.

If, by week 6, 1.5 mm of tooth movement had occurred based on both the radiographic and caliper measurements, the appliance was made passive. Less than 1.5 mm of tooth movement had occurred after 6 weeks in four dogs. For them, the appliance was made passive at week 7. The appliance was made passive by removing the active Beta Titanium wire and 2nd premolar band, and bonding a completely passive 0.030" stainless steel wire to the 1st, 2nd, and 3rd premolars. The passive wire was bonded using Assure Universal Bonding Resin (Reliance, Itasca, IL) and Transbond XT Light Cure Adhesive (3M Unitek, Monrovia, CA). Occlusal radiograph, caliper measurements, and photographs were then obtained. This was followed by a 3-week consolidation period, during which no tooth movement was performed.

To identify regions of new bone formation, fluorescent bone labels were administed three times to each dog at two-week intervals (Table 1). In four dogs, the dyes were administed at weeks 3, 5, and 7. In three dogs, the dyes were administered at weeks 4, 6, and 8. The dye schedule varied to provide for greater information on patterns of bone formation. Dyes were administered in the sequence: Calcein, Alizarin, Calcein (Calcein, 10 mg/kg, MP Biomedicals, Santa Ana, CA; Alizarin complexone, 20 mg/kg, Alfa Aesar, Ward Hill, MA).

Final Records and Sacrifice

Following the 3-week consolidation period, final records were obtained and animals were sacrificed. The animals were first sedated with the previously described Ketamine and Xylazine cocktail. Appliances were removed and the teeth were polished.

Records consisted of occlusal and periapical radiographs, caliper measurements, periodontal probing depths, photographs, and alginate impressions. Surgical plane anesthesia was then confirmed by checking reflexes. The animals were sacrificed by cannulation of both common carotid arteries, severing of the external jugular veins, and injection of 2 cc Beuthanasia D intracardially. Upon cessation of heart function, 1.5 liters of saline followed by 1 liter of 4% paraformaldehyde was flushed through the cannulas. The maxilla was harvested and stored in 4% paraformaldehyde at 4°C.

Data Analysis

Radiographic and Model Measurements

Occlusal radiographs were imported into ViewBox 4 (dHAL Software, Kifissia, Greece) and calibrated for size using a 20 mm radiographic ruler (Figure 3A). A sagittal reference line was drawn that passed midway through the two palatal miniscrews. From this reference line, perpenicular distances to the amalgam markers on the canine, 2nd premolar, and 4th premolar were measured. Measurements were repeated on a separate day. Intraclass correlations of 0.971, 0.998, and 0.999 were obtained from replicated radiographic measurements of canines, 2nd premolars, and 4th premolars, respectively.

To quantify tipping, the initial and final maxillary models were laser scanned using Ortho Insight 3D (Motion View Software, LLC, Chattanooga, TN). Three landmarks were digitized bilaterally on the 2nd premolars, including the most occlusal midline point on the palatal rugae adjacent to the tooth, the most cervical point on the palatal aspect of the tooth, taken at its mesio-distal center, and the cusp tip (Figure 3B).

The angle connecting the three points was measured on both initial and final models, and tipping was calculated as their angular difference. Points and angles were plotted and measured on three separate days and then averaged. Replicate analysis for tipping produced an intraclass correlation of 0.982.

MicroCT Measurements

The overlying soft tissues were kept intact and the maxilla was sectioned to produce blocks approximately 26 mm wide that included the 1st, 2nd, and most of the 3rd premolars. The blocks were approximately 19 mm high occlusoapically, including 3-4 mm of bone apical to the root tip. The apical bone cut was made parallel to the occlusal plane. The blocks were mounted into microCT tubes with an internal diameter of 27 mm. The apical bone surface was inserted first, parallel to the base of the tube, and stabilized with foam. The foam and specimens were kept immersed in 0.5% paraformaldehyde and the tubes were firmly sealed with Parafilm (Pechiney Plastic Packaging Company, Chicago, IL) to prevent drying. The tubes were loaded into the μCT 35 Desktop MicroCT scanner (Scanco Medical, Brüttisellen, Switzerland) and scanned at 30 μm resolution, 55 kVp, 145 μA, and 600 ms integration time.

Three-dimensional reconstructions, as well as two-dimensional slices (30 μm thick), were generated using the Scanco MicroCT v.6.0 software. For 3-D reconstruction, the grayscale images were smoothed by a Gausian filter with a sigma value of 0.9 and support value of 1. The threshold boundaries for the scans were set between 260 and 1,000 Hounsfield units.

The following linear measurements were taken from 2-D axial slices of both the experimental and control premolars:

1) Buccal bone height (BBH), to measure dehiscence, measured from the most lingual aspect of buccal bone at the level of the apex to the mesio-distal center of the buccal bone crest on both mesial and distal roots.
2) Total tooth height (TTH), to measure root resorption, measured from the cusp tip to the root apex.
3) Total root height (TRH), to measure root resorption, measured from the center of the pulpal canal at the level of the furcation to the root apex.
4) Buccal bone thickness (BBT), an indirect measure of tooth movement, measured from the most lingual to the most buccal aspect of buccal bone at the cervical (measured from the middle of the root, 3 mm apical to the level of the furcation), mid (measured from the middle of the root, exactly halfway between the cervical and apical measurements), and apical (measured at the tip of the apex) levels.

All measurements were taken twice, on different days, and averaged. Intraclass correlation of the replicated microCT measurements ranged from 0.973 to 0.999.

Histology

After microCT scanning, the 1st and 3rd premolars were removed from the block specimens, along with the bone more than 2 mm from the root apex. The 2nd premolars were sectioned so that the mesial and distal roots could be processed separately. Each

experimental root was randomly assigned for fluorescence microscopy or decalcified staining (Table A2). Of the control samples, two were prepared for staining and five were prepared for fluorescence microscopy. They were also randomly allocated.

The specimens analyzed with fluorescence microscopy were fixed in 4% paraformaldehyde, dehydrated in graded alcohol, embedded in methyl methacrylate, and allowed to polymerize. Blocks were sectioned with an IsoMet diamond saw (Beuhler, Houston, TX) at a thickness of approximately 150-200 μm. Sections were made in a coronal plane, parallel to the long axis of the root, and then polished to a thickness of about 75-100 μm. Slides were then examined and scanned using the Leica TCS SP5 confocal microscope (Leica Microsystems, Buffalo Grove, IL).

Specimens prepared for staining were decalcified in 0.5 M EDTA, dehydrated in graded alcohol and lastly butyl-alcohol, and infiltrated and embedded in paraffin blocks. The blocks were hardened on a cold plate and then sectioned with a microtome parallel to the long axis of the root at a thickness of 5-10 μm. The sections were mounted to glass slides. Hematoxylin and Eosin (H&E) and Tartrate Resistant Acid Phosphatase (TRAP) stainings were performed, as well as Bone Sialoprotein (BSP) immunostaining.

Statistical Analyses

Statistical analyses were performed with SPSS 22.0 software (SPSS Inc., Chicago, IL). The data was normally distributed. Therefore, central tendencies and dispersions were described using means and standard errors. Paired T-tests were used for the group comparisons. Statistical significance was set at $p<0.05$.

RESULTS

Two of the dogs had one appliance failure each during the experiment. All necessary repairs were performed within 24 hours of appliance breakage (Table A3). One of the dogs had a loose palatal miniscrew, which was removed and replaced upon discovery at week 4 records.

Tooth Movements

Caliper measurements showed (Figure 4A) statistically significant (p=0.030) buccal movement (1.41 mm) of the 2nd premolars during the active tooth movements (T0 to T1) and non-significant (p=0.092) lingual movement (0.24 mm) during consolidation (T1 to T2), for a total movement of 1.17 mm (p=0.026). Radiographic measurements also showed that the experimental 2nd premolars moved (1.42 mm) significantly (p=0.025) in a buccal direction between T0 to T1, and then slightly (0.11 mm), but not significantly (p=0.099), in a lingual direction from T1 to T2. A total of 1.31 mm buccal movement was observed radiographically (Figure 4B). There was no significant difference between the caliper and radiographic measurements of 2nd premolar tooth movement, either for the changes between T0 to T1 (p=0.0851) or for the changes between T1 to T2 (p=0.260). At the end of consolidation, the experimental 2nd premolars had tipped 3.96°, which was not statistically significant (p=0.069). The control premolars tipped 0.5°, which was also not statistically significant (p=0.448) (Figure 5).

Radiographic measurement of inter-canine width did not change during active tooth movement or consolidation. Inter-4th premolar width increased a non-significant 0.1 ± 0.6 mm during active expansion and did not change during consolidation.

Forces and Periodontal Measurements

The force decay of the wires between two-week timepoints ranged from 15-20 grams (Figure 6). No pre-treatment to post-treatment differences were noted for probing depths.

MicroCT

The 3-D image reconstructions showed marked dehiscences on the mesial and distal roots of the experimental 2nd premolars of Dogs A, C, and G (Figure 7). The other four dogs showed slight dehiscences. The experimental 2nd premolar of Dog G also demonstrated extrusion. Fenestration of the distal root tip of the 2nd premolar of Dog F was also observed. The control 2nd premolars showed no dehiscences or fenestrations.

Buccal bone height (BBH) of the mesial root was significantly ($p=0.047$) shorter (4.86 mm) on the experimental than on the control side (6.87 mm). The BBH of the distal roots for the experimental (3.41 mm) and control (5.57 mm) sides were also significantly different ($p=0.020$) (Figure 8). Post-experimental total tooth height (TTH) and total root height (TRH) demonstrated no statistically significant differences between the experimental and control sides (Figure 9).

At the coronal level (i.e. 3 mm apical to the furcation), buccal bone thickness (BBT) over the experimental roots was significantly thinner (0.16 mm – 0.17 mm) than BBT over the control teeth (Figure 10). At the mid-root level, BBT was 0.26 mm – 0.45mm thinner on the experimental than control side. BBT differences between sides were greatest at the apical level, approaching 0.47 mm and 0.39 mm for the mesial and distal roots, respectively, though not statistically significant.

Histology

Fluorescent imaging of the control 2nd premolar all showed mineralization (i.e. green and red fluorescent bands) within osteons of the alveolar bone (Figure 11A). Minimal to no fluorescent labeling was evident along the periosteal and PDL surfaces of alveolar bone in all the control specimens. In contrast, the 2nd premolar demonstrated new bone formation along the periosteal and PDL surfaces of the buccal plate (Figure 11B). The PDL surface of the buccal plate showed a thin green band of new bone likely laid down during the consolidation phase when the last Calcein (green) die was administered. The PDL surface of the palatal bone demonstrated thicker bands of new bone formation. Similar thickness along the coronoapical extent indicated tooth translation. Green and red bands along the root surfaces of the experimental teeth showed new cementum formation (Figure 12A).

H&E staining of coronal sections from the experimental 2nd premolars demonstrated an aggregation of active osteoblasts along both the periosteal and PDL surfaces of the buccal plate (Figure 12B), demonstrating new bone formation. Buccal

bone demonstrated bone lining cells (quiescent osteoblasts) along the periosteal and PDL surfaces of buccal bone in the control section (Figure 13A). Sharpey's fibers were more organized and less numerous in the experimental sections. Few active osteoblasts were evident along the PDL surface of the buccal plate on the control side. The experimental sections demonstrated a band of active osteoblasts extending along the periosteal surface of the buccal plate covering an osteoid surface, soon to be lamellar bone (Figure 13B). Cement lines were evident within the buccal plate of the experimental sections, signifying bone remodeling. Sharpey's fibers were thicker and more numerous in the experimental sections. Experimental sections demonstrated a bone-like matrix on the periosteal and PDL surfaces of the buccal plate, as well as in a region extending coronally from the bone crest. These regions showed increased osteoblast activity, suggesting that new bone will form in these regions (Figure 14B).

TRAP staining from the experimental 2nd premolars demonstrated abundant osteoclast activity along the periosteal surface of the buccal plate (Figure 14). Controls demonstrated fewer TRAP positive cells on the periosteal surface of the buccal plate (Figure 15). Immunostaining for bone sialoprotein (BSP) demonstrated a thick band of new bone formation on the periosteal surface of the buccal plate on the experimental side and a much thinner band on the control side (Figure 16). Thin bands of new bone were also present along the PDL surfaces of the buccal plates in both experimental and control sections.

DISCUSSION

It is possible to buccally translate maxillary premolars with minimal tipping. In the present study, the experimental 2nd premolars tipped 4 degrees, comparable to the 2.2 degrees of tipping reported with the same cantilever system.[60] Similar forces located at the mid crown level produce substantially more tipping. Using a dog model, Kraus et al. reported 15.8 degrees of tipping after applying 81 to 179 grams of force for nine weeks.[4] In humans, 11.7 to 13.5 degrees of tipping have been reported for treatments applying light expansion forces.[52,70] Because the force was applied near the estimated center of resistance in the present study, it was possible to minimize the tipping and produce mostly translation. In addition to the force system used in the present study, the symmetric bands of new bone formation along the palatal surface of the PDL also indicate tooth translation. In contrast, the same coronal orientation of the maxillary 2nd premolars in the study by Kraus et al. demonstrated a greater thickness of new bone on the palatal PDL surface near the crown, and gradually thinner amounts apically.[4] The lack of significant differences in changes in bone thickness in the present study at the coronal, mid-root, or apical levels provides further evidence of tooth translation.

The experimental 2nd premolars were expanded an average of 1.4 mm over 6-7 weeks, which compares favorably to previously reported lateral movements that controlled for tipping. Capps et al. demonstrated 1.6 mm and 1.8 mm of tooth movement of the lingual and buccal premolar cusps, respectively, in human subjects after 9 weeks.[60] Other studies with human subjects have reported rates of buccal tooth movement of single, isolated teeth ranging from 2.7 to 7.1 mm over 7 weeks,[46,70] but

buccal crown tipping was not controlled. Similarly, Kraus et al. demonstrated 3.5 mm of buccal expansion in the dog model, along with significant tipping.[4] Interestingly, the rate of lateral tooth movement in this study was similar to the typical mesio-distal tooth movement rate of 1 mm per month.[71-74] Since cortical bone is denser than trabecular bone, mesio-distal tooth movements should be faster than buccal movements. The similarities in rates may signify that the type of tooth movement (translation versus tipping) is important in determining the rate of tooth movement.

Even though tipping was minimal, dehiscences of the buccal plate occurred. In the current study, approximately 2 mm of dehiscence occurred. There was no correlation between the amount of tipping and the differences in bone heights between experimental and control teeth, suggesting that the dehiscences were not due to tipping. Capps et al. also reported decreases in buccal bone height after premolars had been translated buccally with little to no tipping.[60] This suggests that factors other than tipping must be involved.[1,4,52,56] One possible factor is bone thickness. As a tooth is translated through buccal bone, its thickness decreases. Since crestal bone is very thin, it is possible that the premolar translated through that region of bone before it could adapt. It is also possible that the crestal bone was strained beyond its adaptive capabilities, resulting in bone loss due to an excess of microfractures.[38] The crestal bone in the present study was much thinner than the mid root and apical bone. Thinner regions of the buccal plate might be expected to experience more strain (ie. deformation) than thicker regions.

Most importantly, new buccal bone formed as a result of lateral tooth movement. New bone formation was apparent in the fluorescent, H&E, and bone sialoprotein

immunosstained sections. Experimental sections showed three distinct bands of triple labeling along the periosteal surface, signifying new bone formation. In contrast, the control sections demonstrated faint or no new bone formation along the periosteal surface, indicative of the normal bone turnover that occurs in the absence of active tooth movement. The H&E sections also showed osteoblasts lining the periosteal surface only on the experimental side. Moreover, bone sialoprotein immunostaining demonstrated much larger areas of new bone formation on the experimental than control side. Kraus et al. were among the first to experimentally demonstrate buccal bone formation after lateral tooth movements.[4] Using CBCT, Capps et al. showed new buccal bone formation (0.51 mm) after 9 weeks of buccal tooth movements, as did Corbridge after quad-helix expansion.[59,60] New bone formation is an adaptive response to the increased forces associated with tooth movement. As explained by Frost, bone mass and bone strength increase when strain reaches a certain threshold.[38,62] Bone regulates the amount of strain it is exposed to by modifying its structure.[19] This explains why increased stress on the dentoalveolar complex increases the thickness of the alveolar cortex.[61,75]

The periosteal surface of the buccal plate exhibited both modeling and remodeling. Modeling was clearly demonstrated in the histomorphometric sections by the linear pattern of new bone formation apparent on the periosteal surface. Edwards also reported a modeling process associated with the retraction of incisors. Implants placed in the palatal cortex were “left behind” and surrounded by cancellous bone after tooth movement, signifying that the palatal cortical plate moved lingually by osseous apposition. In the current study, bone was modeled along the buccal plate to

accommodate the lateral displacement of the premolars. Histology in the present study also demonstrated remodeling along the periosteal surface of the buccal plate. TRAP staining demonstrated markedly increased osteoclastic activity on the periosteal surface of the buccal plate of the experimental sections compared to the controls. It is likely that osteoclasts were breaking down new bone formed on the buccal plate in order to form more mature bone. Furthermore, the 3D μCT reconstructions showed that buccal bone was rougher on the experimental than control side, which signifies new immature bone remodeling into to mature bone over time.

Translatory tooth movements in the present study resulted in significant dehiscenses on the buccal surfaces, as well as significant decreases in buccal bone thicknesses. Previous studies demonstrated similar results, with bone gain not keeping up with bone loss.[4,60] It is possible that the amount of strain experienced during the tooth movement phase was in the "pathologic" state described by Frost.[38] At high levels of strain, more bone is resorbed than added, since repair cannot keep up with the microfractures that occur, resulting in a net decrease in bone. Another possible explanation is that the disuse of bone during the consolidation phase may limit new bone formation. Because the dogs were on a soft diet thoughout the study, as well as during the 3-week consolidation period, the alveolar bone may not have been strained sufficiently by biting and chewing forces. It could be argued that normal masticatory forces would produce greater bone strain and stimulate more new bone formation.

As expected, new bone was also formed on the bone lining the palatal surface of the PDL, as well as on the PDL surface of the buccal plate. Epker and Frost showed that

stretching of the PDL fibers causes bone bending in this tension zone, resulting in apposition of bone.[37] Baumrind explained that reorganization occurs in the lamina dura as well as on the surfaces of all the trabeculae within that region of bone that is strained.[30] This bone turnover and renewal allows for the dentoalveolar complex to adapt to orthodontic strains.

New bone formation continues after the consolidation period. The experimental H&E sections in the present study demonstrated a bone-like matrix on the periosteal and PDL surfaces of the buccal plate, as well as in a region extending coronally from the crestal bone. These regions showed increased osteoblast activity, indicating that new bone will form in these areas. This suggests that new bone formation lags behind bone resorption associated with tooth displacement. Others have previously shown that bony defects, created by buccal tipping of teeth, can be repaired after the teeth are moved back into their original positions.[55,56] For example, Wainwright, who moved a root tip through the buccal plate and then retained the tooth for 4 months, showed new bone nearly covering the root apex and cortical plate directly surrounding the area of perforation.[54] It is likely that bone was being modeled over a more buccally positioned root in response to strains placed on the buccal cortex.

CHAPTER III

CONCLUSIONS

The present study found that it is possible to buccally translate maxillary premolars with minimal tipping. Even when tipping was minimized, approximately 2 mm of dehiscence of the buccal plate occurred with translatory tooth movements. Furthermore, new bone is formed on the periosteal side of the buccal plate in response to translatory tooth movements. Despite bone gain, a net decrease in buccal bone thickness occurs with buccal translatory tooth movements. Lastly, new bone formation continues to occur after the consolidation period.

REFERENCES

1. Steiner GG, Pearson JK, Ainamo J. Changes of the marginal periodontium as a result of labial tooth movement in monkeys. J Periodontol 1981;52:7.

2. Reitan K. Tissue behavior during orthodontic tooth movement. Am J Orthod 1960;46:881-890.

3. Graber LW, Vanarsdall RL, Vig KWL. Orthodontics: Current Principles and Techniques, 5e. Philadelphia: Elsevier Mosby; 2012.

4. Kraus CD, Campbell PM, Spears R, Taylor RW, Buschang PH. Bony adaptation after expansion with light-to-moderate continuous forces. Am J Orthod Dentofacial Orthop 2014;145:655-666.

5. Proffit WR, Fields HW, Moray LJ. Prevalence of malocclusion and orthodontic treatment need in the United States: estimates from the N-HANES III survey. Int J Adult Orthodon Orthognath Surg 1998;13:97-106.

6. Brook PH, Shaw WC. The development of an index for orthodontic treatment priority. Eur J Orthod 1989;11:309-320.

7. Brunelle JA, Bhat M, Lipton JA. Prevalence and distribution of selected occlusal characteristics in the US population, 1988-1991. J Dent Res 1996;75 Spec No:706-713.

8. McNamara JA, Brudon WL. Orthodontic and Orthopedic Treatment in the Mixed Dentition. Needham Press; 1993.

9. Brieden CM, Pangrazio-Kulbersh V, Kulbersh R. Maxillary skeletal and dental changes with Frankel appliance therapy. The Angle Orthodontist 1984;54:226-232.

10. Thorne NH. Experiences on widening the median maxillary suture. Eur J Oral Soc Trans 1956;31:279-290.

11. Hicks EP. Slow maxillary expansion: A clinical study of the skeletal versus dental response to low-magnitude force. Am J Orthod 1978;73:121-141.

12. Proffit W, Fields H, Sarver D. Contemporary Orthodontics, Fourth Edition. St Louis, Missouri: Mosby Elsevier; 2007.

13. Baccetti T, Franchi L, Cameron CG, McNamara JA. Treatment timing for rapid maxillary expansion. Angle Orthod 2001;71:343-350.

14. Adkins MD, Nanda RS, Currier GF. Arch perimeter changes on rapid palatal expansion. Am J Orthod Dentofacial Orthop 1990;97:194-199.

15. Germane N, Lindauer SJ, Rubenstein LK, Revere JH, Jr., Isaacson RJ. Increase in arch perimeter due to orthodontic expansion. Am J Orthod Dentofacial Orthop 1991;100:421-427.

16. Motoyoshi M, Hirabayashi M, Shimazaki T, Namura S. An experimental study on mandibular expansion: Increases in arch width and perimeter. Eur J Orthod 2002;24:125-130.

17. Weinman J, Sicher H. Bone and Bones. Fundamentals of Bone Biology. St. Louis: The C. V. Mosby Company; 1955.

18. Roberts WE, Roberts JA, Epker BN, Burr DB, Hartsfield JK. Remodeling of Mineralized Tissues, Part I: The Frost Legacy. Semin Orthod 2006;12:216-237.

19. Martin RB, Burr DB, Sharkey NA. Skeletal Tissue Mechanics. New York: Springer; 1998.

20. Frost HM, Villanueva AR, Roch H, Stanisavljevic S. Tetracycline bone labeling. J New Drugs 1961;1:206-216.

21. Parfitt AM. The physiologic and clinical significance of bone histomorphometric data. In: Becker RR, editor. Bone Histomorphometry Techniques and Interpretation. Boca Raton: CRC Press; 1983. p. 143-223.

22. Graber TM, Vanarsdall RL. Orthodontics: Current principles and techniques. St. Louis: Mosby; 1994.

23. Nanci A. Ten Cate's Oral Histology: Development, Structure, and Function. Mosby; 2012.

24. Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop 2006;129:469 e461-432.

25. Sandstedt C. Einige Beiträge zur Theorie der Zahnregulierung. Nordisk Tandläkare Tidskrift 1905;6:1-25, 141-168.

26. Schwarz AM. Tissue changes incidental to orthodontic tooth movement. International Journal of Orthodontia, Oral Surgery and Radiography 1932;18:331-352.

27. Kardos TB, Simpson LO. A new periodontal membrane biology based upon thixotropic concepts. Am J Orthod 1980;77:508-515.

28. Yee JA, Kimmel DB, Jee WS. Periodontal ligament cell kinetics following orthodontic tooth movement. Cell Tissue Kinet 1976;9:293-302.

29. Reitan K. Some factors determining the evaluation of force in orthodontics. Am J Orthod 1957;43:32-45.

30. Baumrind S. A reconsideration of the propriety of the "pressure-tension" hypothesis. Am J Orthod 1969;55:12-22.

31. Zander HA, Muhlemann HR. The effect of stresses on the periodontal structures. Oral Surg Oral Med Oral Pathol 1956;9:380-390.

32. Grimm FM. Bone bending, a feature of orthodontic tooth movement. Am J Orthod 1972;62:384-393.

33. Jansen M. On bone formation: Its relation to tension and pressure. London: Longmans; 1920.

34. Frost HM. The Laws of Bone Structure. Springfield: Thomas; 1964.

35. Zengo AN, Bassett CA, Pawluk RJ, Prountzos G. In vivo bioelectric potentials in the dentoalveolar complex. Am J Orthod 1974;66:130-139.

36. Bassett CA, Becker RO. Generation of electric potentials by bone in response to mechanical stress. Science 1962;137:1063-1064.

37. Epker BN, Frost HM. Correlation of bone resorption and formation with the physical behavior of loaded bone. J Dent Res 1965;44:33-41.

38. Frost HM. Bone "mass" and the "mechanostat": A proposal. Anat Rec 1987;219:1-9.

39. Melsen B. Biological reaction of alveolar bone to orthodontic tooth movement. Angle Orthod 1999;69:151-158.

40. Friedenberg ZB, Andrews ET, Smolenski BI, Pearl BW, Brighton CT. Bone reaction to varying amounts of direct current. Surg Gynecol Obstet 1970;131:894-899.

41. Davidovitch Z, Finkelson MD, Steigman S, Shanfeld JL, Montgomery PC, Korostoff E. Electric currents, bone remodeling, and orthodontic tooth movement. I. The effect of electric currents on periodontal cyclic nucleotides. Am J Orthod 1980;77:14-32.

42. Davidovitch Z, Finkelson MD, Steigman S, Shanfeld JL, Montgomery PC, Korostoff E. Electric currents, bone remodeling, and orthodontic tooth

movement. II. Increase in rate of tooth movement and periodontal cyclic nucleotide levels by combined force and electric current. Am J Orthod 1980;77:33-47.

43. Quinn R, Yoshikawa DK. A reassessment of force magnitude in orthodontics. Am J Orthod Dentofacial Orthop 1985;88:9.

44. Boester CH, Johnston LE. A clinical investigation of the concepts of differential and optimal force in canine retraction. Angle Orthod 1974;44:113-119.

45. Pilon J, Kuijpers-Jagtman A, Maltha J. Magnitude of orthodontic forces and rate of bodily tooth movement. An experimental study. Am J Orthod Dentofacial Orthop 1996;110:8.

46. Owman-Moll P, Kurol J, Lundgren D. Effects of a doubled orthodontic force magnitude on tooth movement and root resorptions. An inter-individual study in adolescents. Eur J Orthod 1996;18:141-150.

47. Ren Y, Maltha JC, Kujpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: A systematic literature review. Angle Orthod 2003;73:86-92.

48. Ren Y, Maltha JC, Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: A mathematic model. Am J Orthod Dentofacial Orthop 2004;125:71-77.

49. von Bohl M, Maltha J, Von Den Hoff JW, Kuijpers-Jagtman AM. Focal hyalinization during experimental tooth movement in beagle dogs. Am J Orthod Dentofacial Orthop 2004;125:615-623.

50. von Bohl M, Maltha J, Von den Hoff JW, Kuijpers-Jagtman A. Changes in the periodontal ligament after experimental tooth movement using high and low continuous forces in beagle dogs. Angle Orthod 2004;74:16-25.

51. Ricketts RM. Bioprogressive therapy as an answer to orthodontic needs Part I. Am J Orthod 1976;70.

52. Cattaneo PM, Treccani M, Carlsson K, Thorgeirsson T, Myrda A, Cevidanes LH et al. Transversal maxillary dento-alveolar changes in patients treated with active and passive self-ligating brackets: A randomized clinical trial using CBCT-scans and digital models. Orthod Craniofac Res 2011;14:222-233.

53. Ruso S. Bone response to buccal tooth movement- with and without flapless alveolar decortication. Department of Orthodontics. Dallas, TX: Texas A&M Baylor College of Dentistry; 2012.

54. Wainwright WM. Faciolingual tooth movement: Its influence on the root and cortical plate. Am J Orthod 1973;64:278-302.

55. Engelking G, Zachrisson BU. Effects of incisor repositioning on monkey periodontium after expansion through the cortical plate. Am J Orthod 1982;82:23-32.

56. Karring T, Nyman S, Thilander B, Magnusson I. Bone regeneration in orthodontically produced alveolar bone dehiscences. J Periodontal Res 1982;17:309-315.

57. Thilander B. Infrabony pockets and reduced alveolar bone height in relation to orthodontic therapy. Semin Orthod 1996;2:55-61.

58. Edwards JG. A study of the anterior portion of the palate as it relates to orthodontic therapy. Am J Orthod 1976;69:249-273.

59. Corbridge JK, Campbell PM, Taylor R, Ceen RF, Buschang PH. Transverse dentoalveolar changes after slow maxillary expansion. Am J Orthod Dentofacial Orthop 2011;140:317-325.

60. Capps CJ, Campbell PM, Benson B, Buschang PH. Can posterior teeth of patients be translated buccally, and does bone form on the buccal surface in response? Angle Orthod 2015;In-Press.

61. Horner KA, Behrents RG, Kim KB, Buschang PH. Cortical bone and ridge thickness of hyperdivergent and hypodivergent adults. Am J Orthod Dentofacial Orthop 2012;142:170-178.

62. Frost HM. Bone's mechanostat: a 2003 update. Anat Rec A Discov Mol Cell Evol Biol 2003;275:1081-1101.

63. Melsen B. Tissue reaction to orthodontic tooth movement -- a new paradigm. European Journal of Orthodontics 2001;23:671-681.

64. Aerssens J, Boonen S, Lowet G, Dequeker J. Interspecies differences in bone composition, density, and quality: potential implications for in vivo bone research. Endocrinology 1998;139:663-670.

65. Buschang PH, Campbell PM, Ruso S. Accelerating Tooth Movement With Corticotomies: Is It Possible and Desirable? Semin Orthod 2012;18:286-294.

66. Bartley MH, Taylor GN, Jee WS. The beagle as an experimental dog. In: Anderson AC, editor. Ames, Iowa: The Iowa State University Press; 1970: p. 189-215.

67. Burstone CJ. The biomechanics of tooth movement. Philadelphia, PA: Lea & Febiger; 1962.

68. Burstone CJ, Pryputniewicz RJ. Holographic determination of centers of rotation produced by orthodontic forces. Am J Orthod 1980;77:396-409.

69. Tanne K, Koenig HA, Burstone CJ. Moment to force ratios and the center of rotation. Am J Orthod Dentofacial Orthop 1988;94:426-431.

70. Lundgren D, Owman-Moll P, Kurol J. Early tooth movement pattern after application of a controlled continuous orthodontic force. A human experimental model. Am J Orthod Dentofacial Orthop 1996;110:287-294.

71. Cho KW, Cho SW, Oh CO, Ryu YK, Ohshima H, Jung HS. The effect of cortical activation on orthodontic tooth movement. Oral Dis 2007;13:314-319.

72. Iino S, Sakoda S, Ito G, Nishimori T, Ikeda T, Miyawaki S. Acceleration of orthodontic tooth movement by alveolar corticotomy in the dog. Am J Orthod Dentofacial Orthop 2007;131:448.e441-448.

73. Samuels RH, Rudge SJ, Mair LH. A clinical study of space closure with nickel-titanium closed coil springs and an elastic module. Am J Orthod Dentofacial Orthop 1998;114:73-79.

74. Nightingale C, Jones SP. A clinical investigation of force delivery systems for orthodontic space closure. J Orthod 2003;30:229-236.

75. Ichim I, Kieser JA, Swain MV. Functional significance of strain distribution in the human mandible under masticatory load: Numerical predictions. Arch Oral Biol 2007;52:465-473.

APPENDIX A

Week / Dog	3	4	5	6	7	8	9	10
A E F		Calcein		Alizarin	Passive	Calcein		Sacrifice
B		Calcein		Alizarin Passive		Calcein	Sacrifice	
D	Calcein		Alizarin		Calcein Passive			Sacrifice
C G	Calcein		Alizarin	Passive	Calcein		Sacrifice	

Table A1. **Experiment Schedule**. Summary of fluorescent dye schedule, passive wire placement, and animal sacrifice.

Dog	Side	Root	Histology Type
A	Experiment (R)	Mesial	Fluorescence
		Distal	NE
	Control (L)	Mesial	NE
		Distal	Fluorescence
B	Experiment (L)	Mesial	H&E, TRAP, and BSP
		Distal	Fluorescence
	Control (R)	Mesial	NE
		Distal	Fluorescence
C	Experiment (R)	Mesial	Fluorescence
		Distal	NE
	Control (L)	Mesial	NE
		Distal	NE
D	Experiment (L)	Mesial	Fluorescence
		Distal	H&E, TRAP, and BSP
	Control (R)	Mesial	NE
		Distal	NE
E	Experiment (L)	Mesial	H&E, TRAP, and BSP
		Distal	Fluorescence
	Control (R)	Mesial	H&E and TRAP
		Distal	Fluorescence
F	Experiment (L)	Mesial	Fluorescence
		Distal	H&E and TRAP
	Control (R)	Mesial	Fluorescence
		Distal	NE
G	Experiment (R)	Mesial	NE
		Distal	Fluorescence
	Control (L)	Mesial	Fluorescence
		Distal	H&E, TRAP, and BSP

Table A2. **Histologic Designations**. Summary of bilateral specimen histologic allocations with corresponding roots. *H&E,* Hematoxylin and Eosin; *TRAP*, Tartrate Resistant Acid Phosphatase; *BSP*, Bone Sialoprotein; *NE,* not evaluated.

Dog	Week	Description of Breakage	Correction
A	1	Canine tube sheared from band	Remade and rebonded
C	2	4^{th} premolar tube sheared from band	Remade and rebonded
G	4	Miniscrew was loose	Replaced miniscrew

Table A3. **Appliance Breakages**. Summary of breakages and repairs during the 9-week experimental duration.

	Variable		Experimental		Control		Diff Prob
			Mean	S.E.	Mean	S.E.	
MicroCT: Buccal Bone Thickness	Apical	Mesial Root	0.92	0.23	1.39	0.21	0.029
		Distal Root	0.33	0.12	0.72	0.07	0.016
	Mid-Root	Mesial Root	0.38	0.10	0.83	0.18	0.009
		Distal Root	0.19	0.08	0.45	0.05	0.022
	Coronal	Mesial Root	0.09	0.05	0.25	0.03	0.011
		Distal Root	0.02	0.02	0.19	0.04	0.002
MicroCT:	BBH	Mesial Root	4.86	0.70	6.87	0.28	0.047
		Distal Root	3.41	0.76	5.57	0.18	0.020
	TRH	Mesial Root	8.63	0.29	8.64	0.28	NS
		Distal Root	7.57	0.21	7.71	0.24	NS
	TTH	Mesial Root	14.71	0.44	14.73	0.42	NS
		Distal Root	13.97	0.35	13.97	0.31	NS

Table A4. **Descriptive Statistics**. MicroCT pre- and post-experimental changes and comparisons.

APPENDIX B

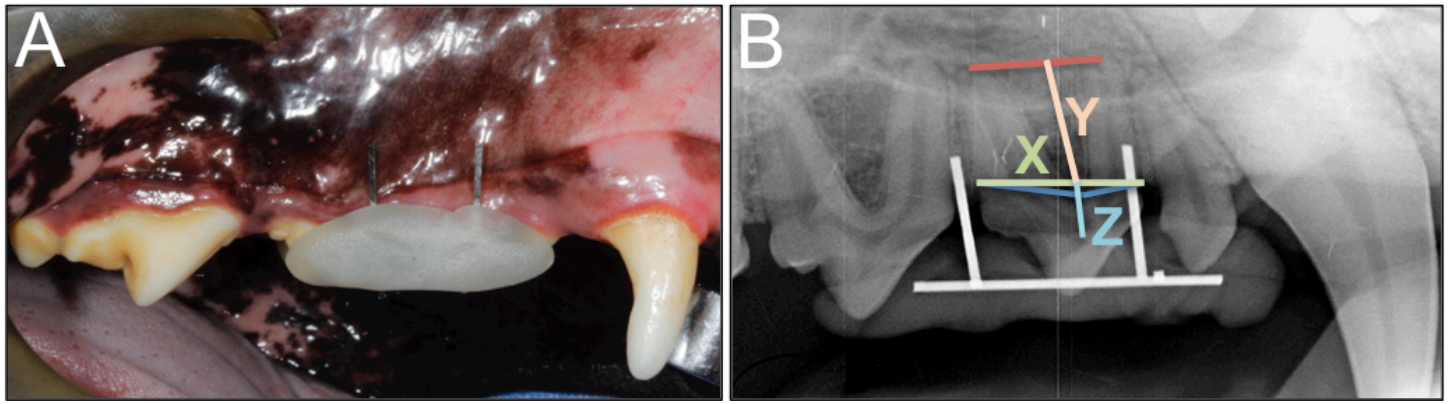

Figure 1. **Measurement of Center of Resistance**. **A**, Custom measurement jig for acquiring periapical radiographs of the experimental teeth. Two 10-mm long stainless steel wires were oriented parallel to the mesial and distal roots of the second premolar for image calibration. **B**, The center of resistance was estimated halfway (50%) between the root apex and alveolar crest. The length of the extension arm from the gingival margin was calculated with the formula: (Y/2) +Z.

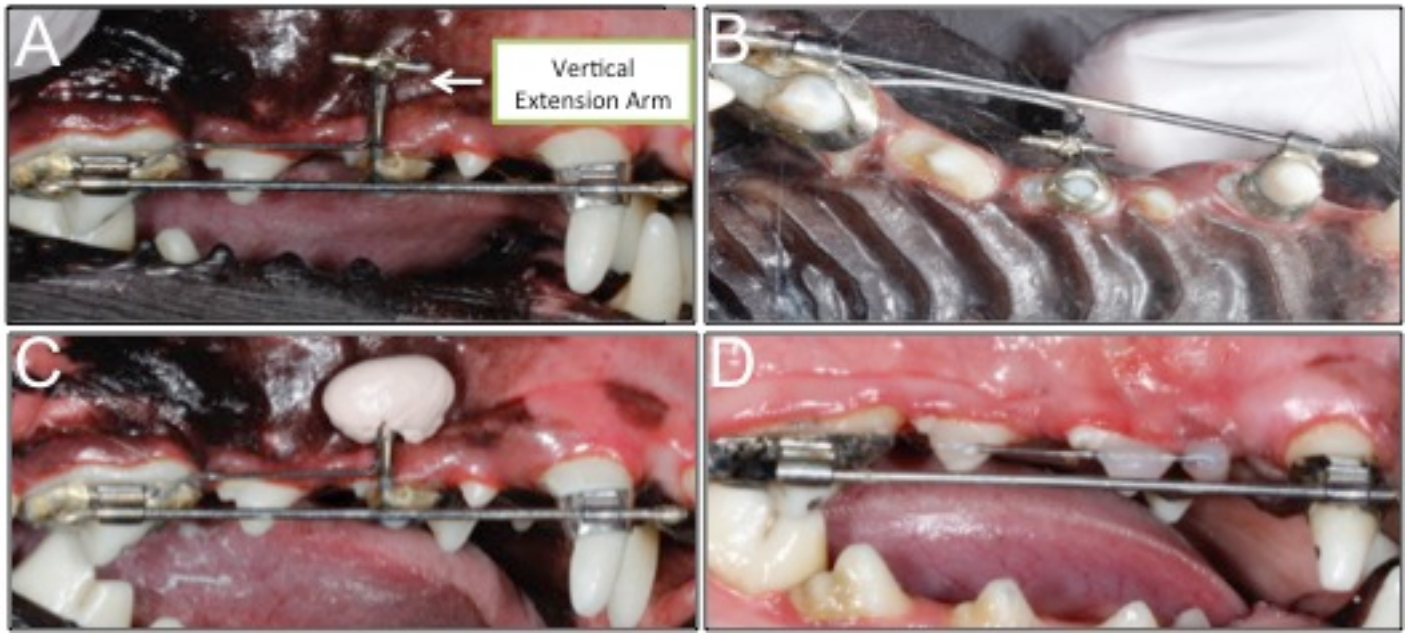

Figure 2. **Appliance Design**. **A** & **B**, Activated appliance consisting of bands on the canine, 2nd premolar, and 4th premolar, along with a passive stainless steel wire connecting the canine and 4th premolar, a vertical extension arm on the 2nd premolar band, and a Beta Titanium wire activated to 100g. The active wire was looped at one end, tied to the apical extent of the vertical arm on the 2nd premolar and engaged through the 4th premolar tube. **C**. Coe-Pak on vertical extension arm for cheek protection. **D**, Passive wire bonded to 1st, 2nd, and 3rd premolars during 3-week consolidation period.

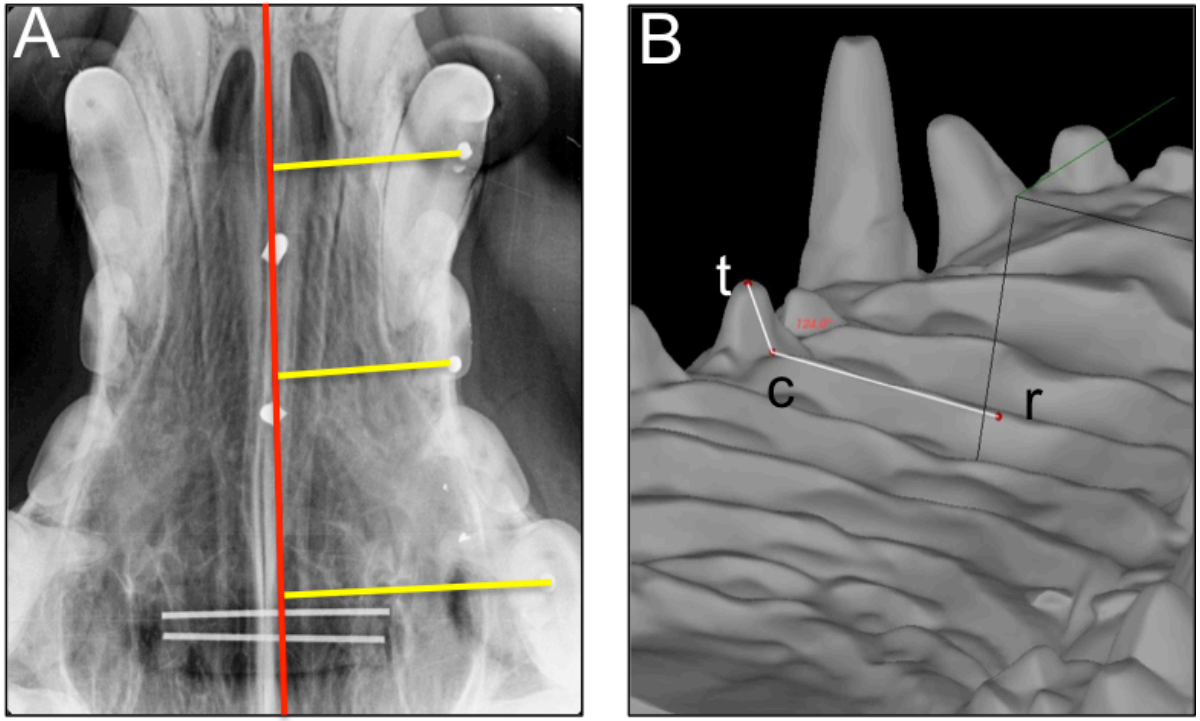

Figure 3. **Radiographic Measurements of Tooth Movement**. **A**, Radiographic measurements of tooth movement: A reference line was constructed through the midpoints of the two palatal implants. The perpendicular distances from amalgam markers on the canine, 2nd premolar, and 4th premolar to the reference line were measured. **B**, Dental casts were laser scanned and digitized to evaluate tipping of the 2nd premolars, calculated as the angular difference between pre- and post-experimental measurements. Angles were defined by connecting the points **r**, the most occlusal midline point on the palatal rugae adjacent to the 2nd premolar, **c**, the most cervical point on the palatal aspect of the 2nd premolar, taken at its mesio-distal center, and **t**, the cusp tip.

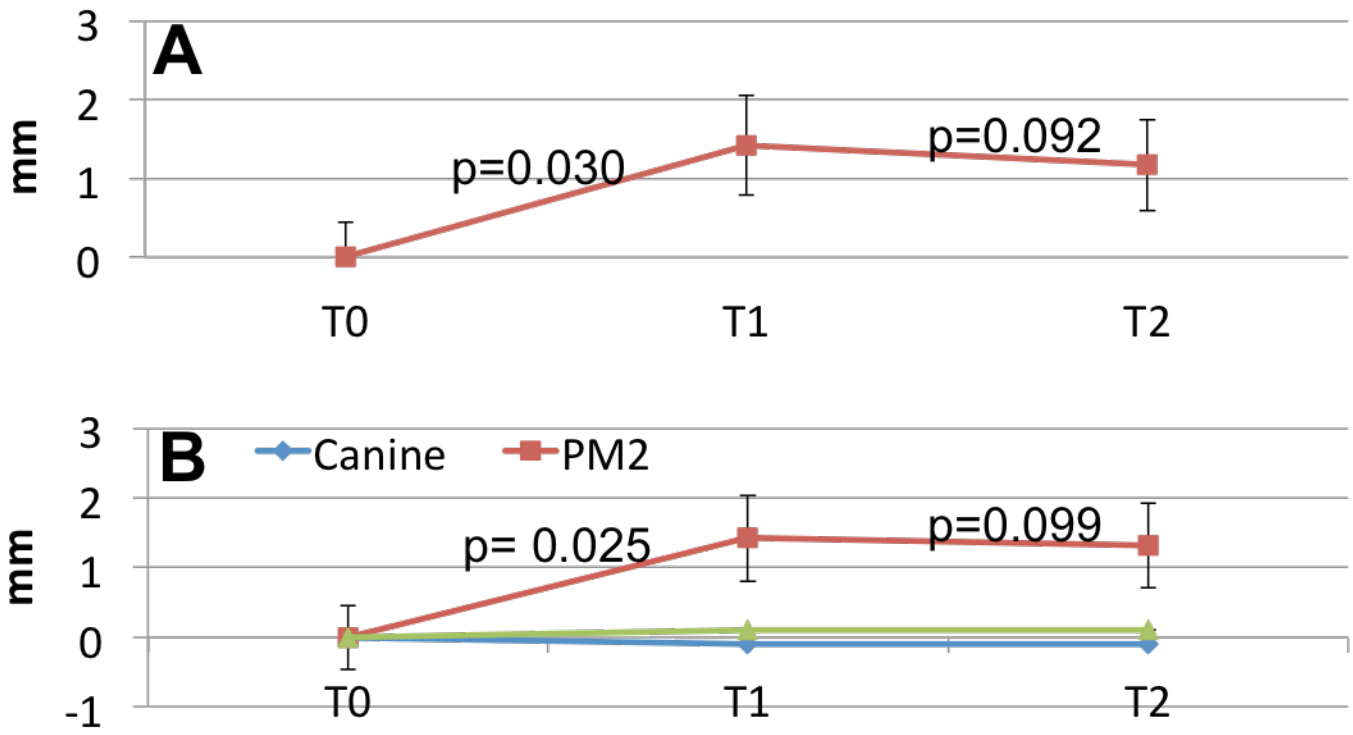

Figure 4. **Intraoral Measurements of Tooth Movement**. **A**, Intraoral caliper measurements of 2nd premolar tooth movement. **T0**, Initial; **T1**, Weeks 6 or 7; **T2**, Weeks 9 or 10 (post-consolidation). **B**, Radiographic measurements of canine, 2nd premolar, and 4th premolar movement. Error bars indicate standard errors of the mean.

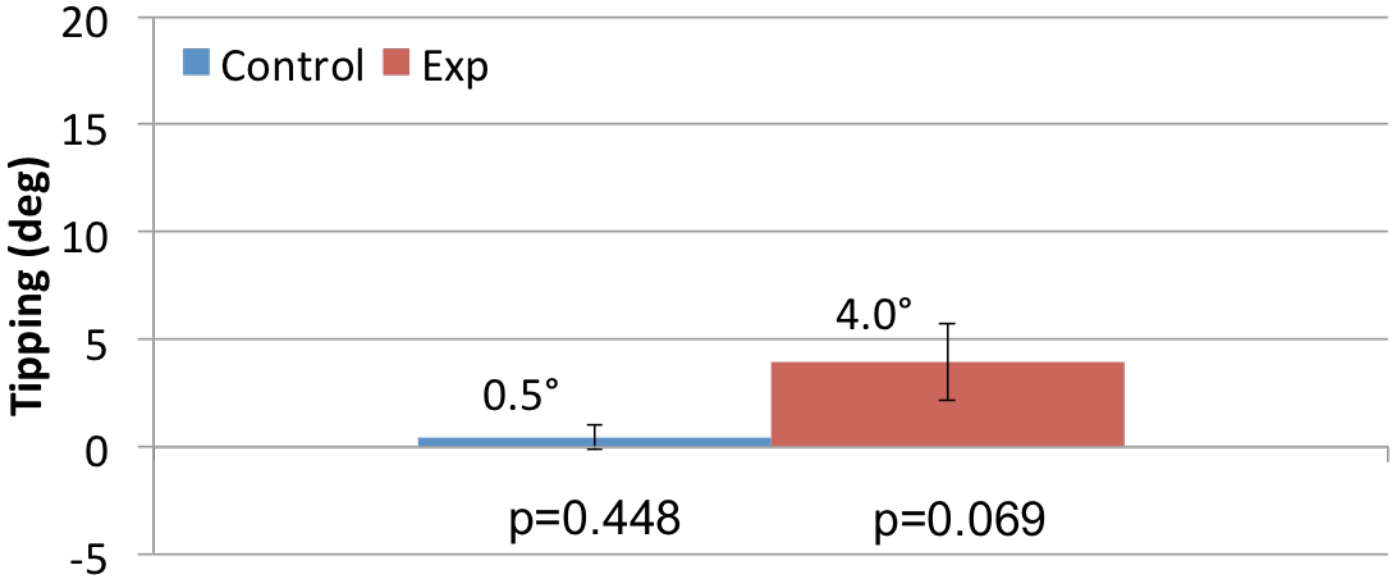

Figure 5. **Tipping Measurements**. Means, standard errors, and probabilities of dental tipping for the control and experimental 2nd premolars.

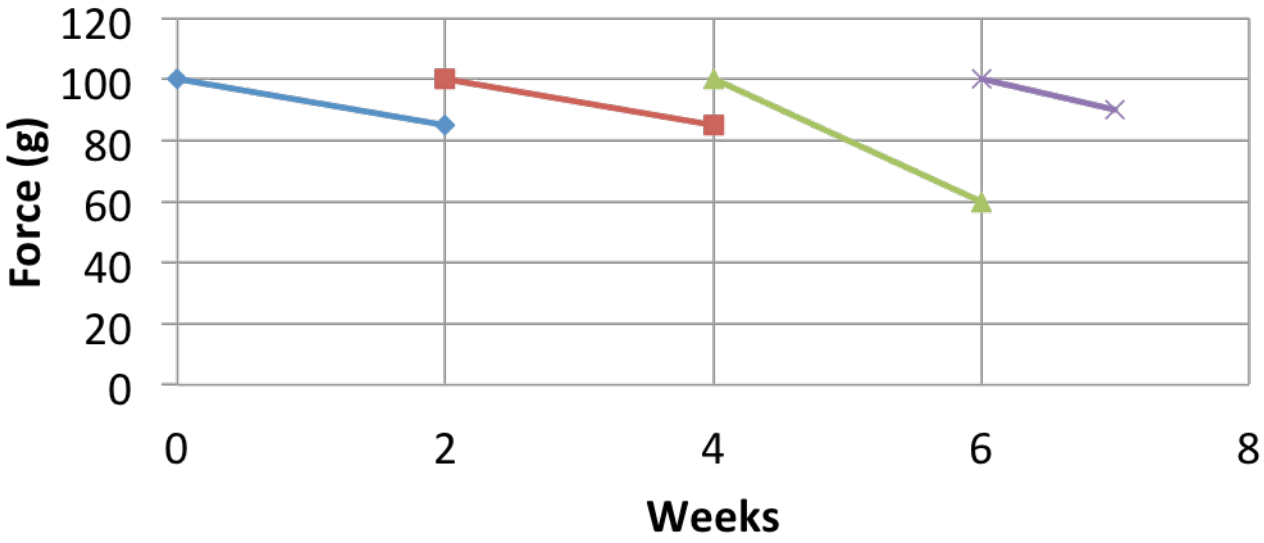

Figure 6. **Force Measurements**. Force Measurements. Average forces, in grams, exerted by the Beta Titanium wires on the 2nd premolars at the end of each time interval. Wires were re-activated to 100 grams using a Correx gram force gauge every two weeks.

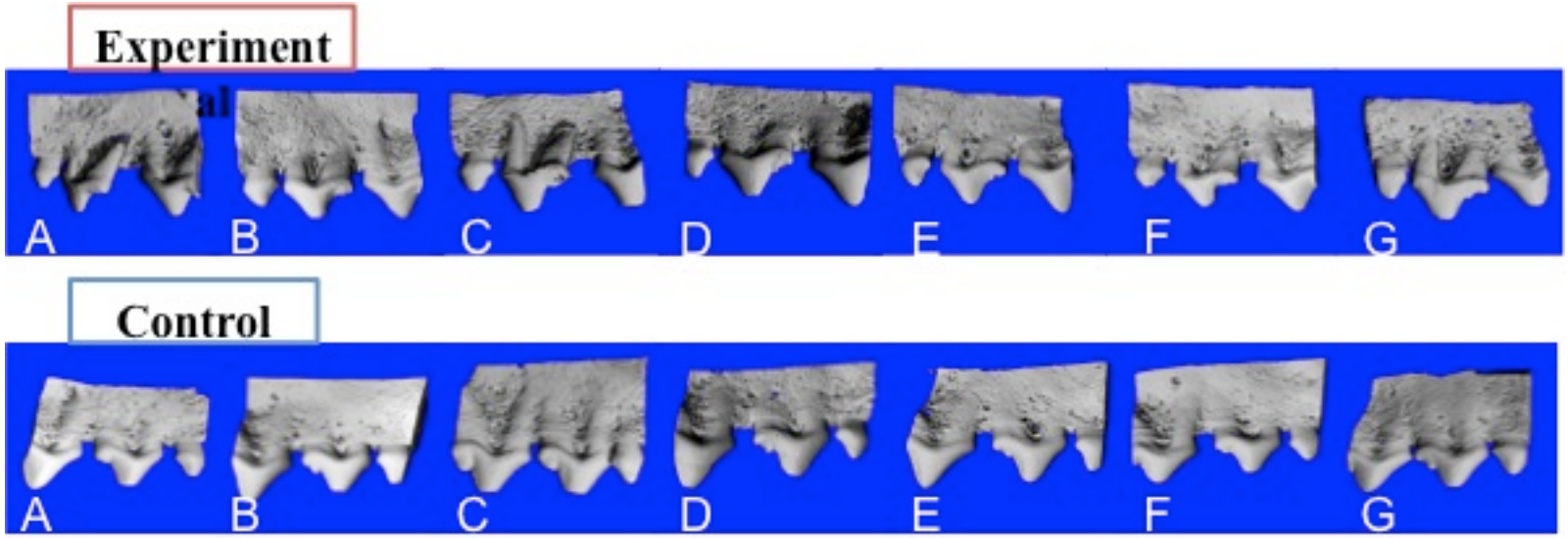

Figure 7. **Three-Dimensional MicroCT Reconstructions**. Buccal views of microCT reconstructions of experimental and control 2nd premolars (middle tooth) with adjacent 1st (single-rooted) and 3rd (double-rooted) premolars. Note extensive dehiscences on the experimental roots of A, C, and G and fenestration of the distal root of F.

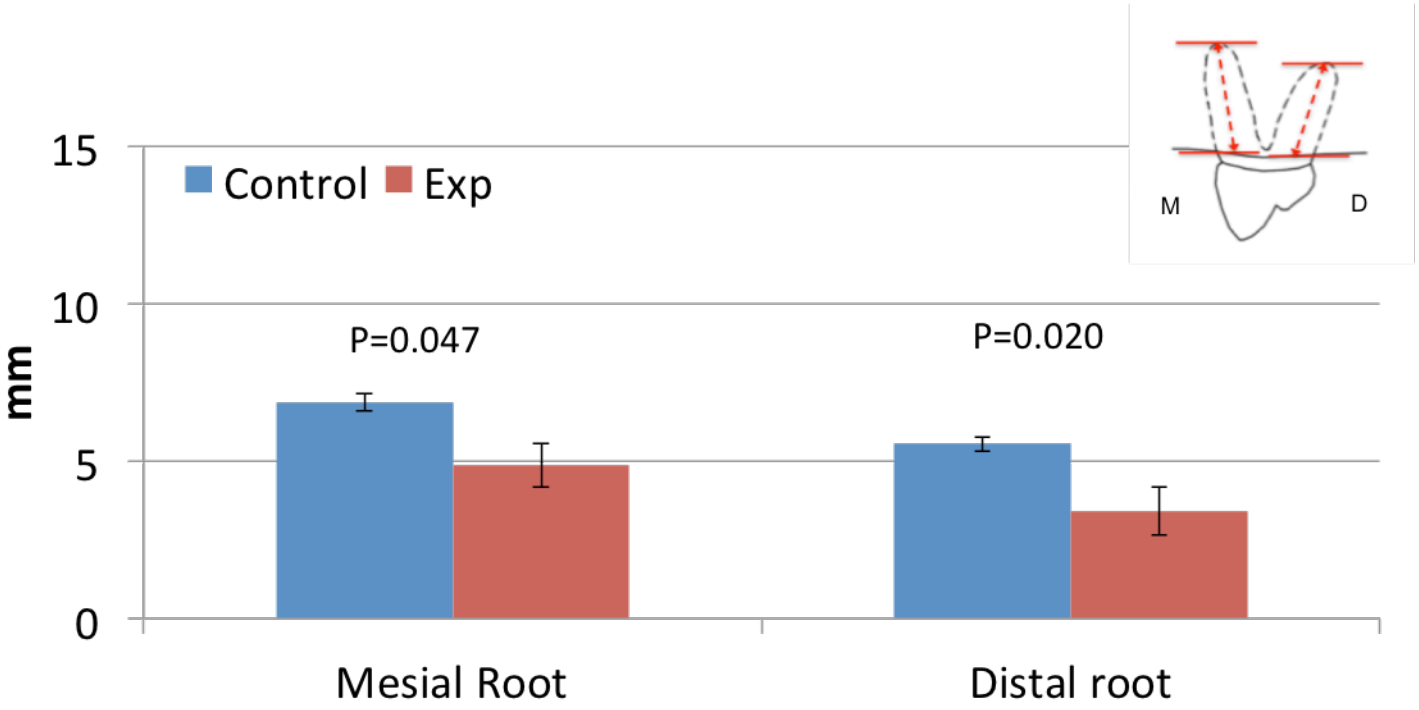

Figure 8. **MicroCT: Buccal Bone Height Measurements**. Buccal bone height measurements, standard errors, and probabilities of side differences (N=7). MicroCT 3-D measurements of vertical bone height measured from the most lingual aspect of buccal bone at the level of the apex to the mesiodistal center of the buccal bone crest on both mesial and distal roots.

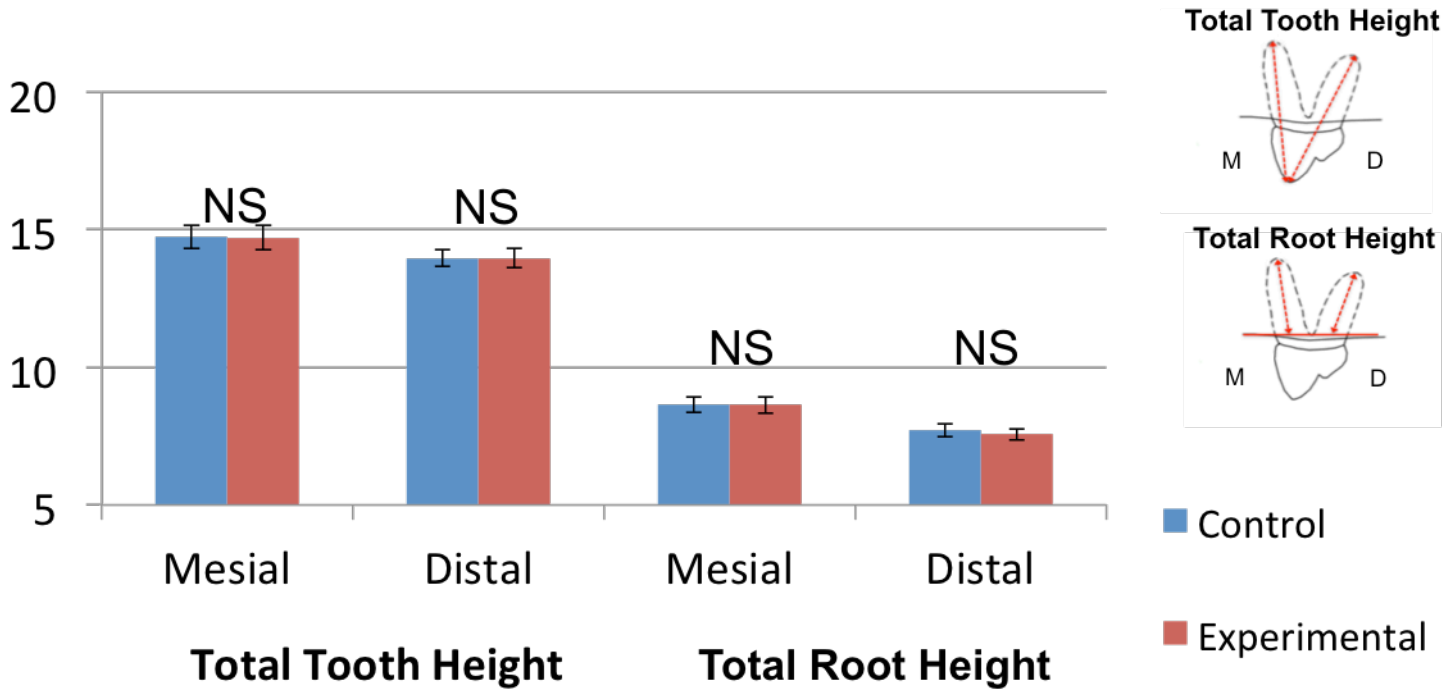

Figure 9. **MicroCT: Total Tooth Height and Total Root Height Measurements**. 3-D MicroCT measures, along with standard errors and probabilities of side differences (N=7) of root resorption: Total tooth height, measured from the cusp tip to the root apex. Total root height, measured from the center of the pulpal canal at the level of the furcation to the root apex. No significant differences were found, indicating that changes in buccal bone height cannot be attributed to root resorption.

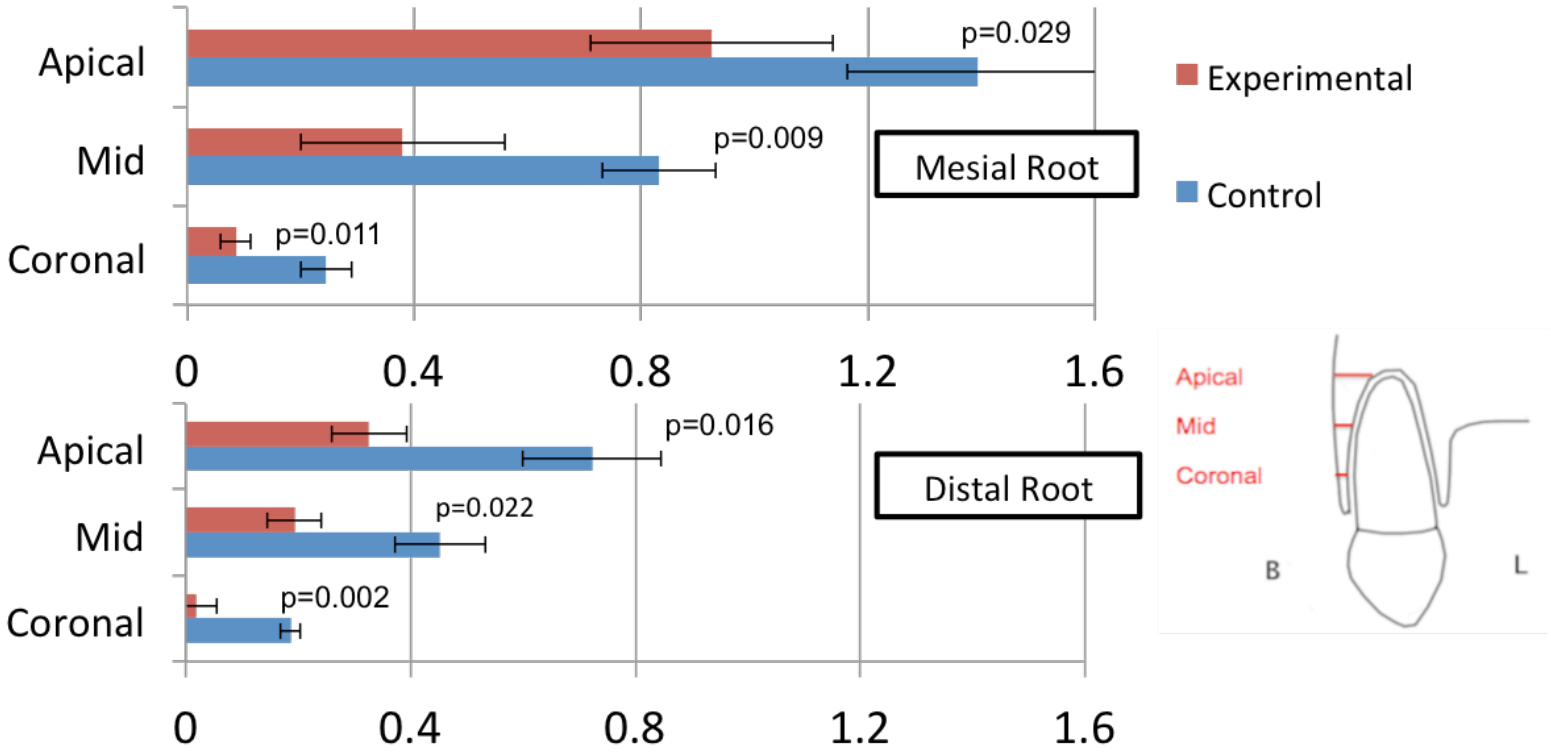

Figure 10. **MicroCT: Buccal Bone Thickness Measurements**. Buccal bone thickness, an indirect measure of tooth movement, measured from the most lingual to the most buccal aspect of buccal bone at the cervical (measured from the middle of the root, 3 mm apical to the level of the furcation), mid (measured from the middle of the root, exactly halfway between the cervical and apical measurements), and apical (measured at the tip of the apex) levels, with standard errors and probabilities of side differences (N=7).

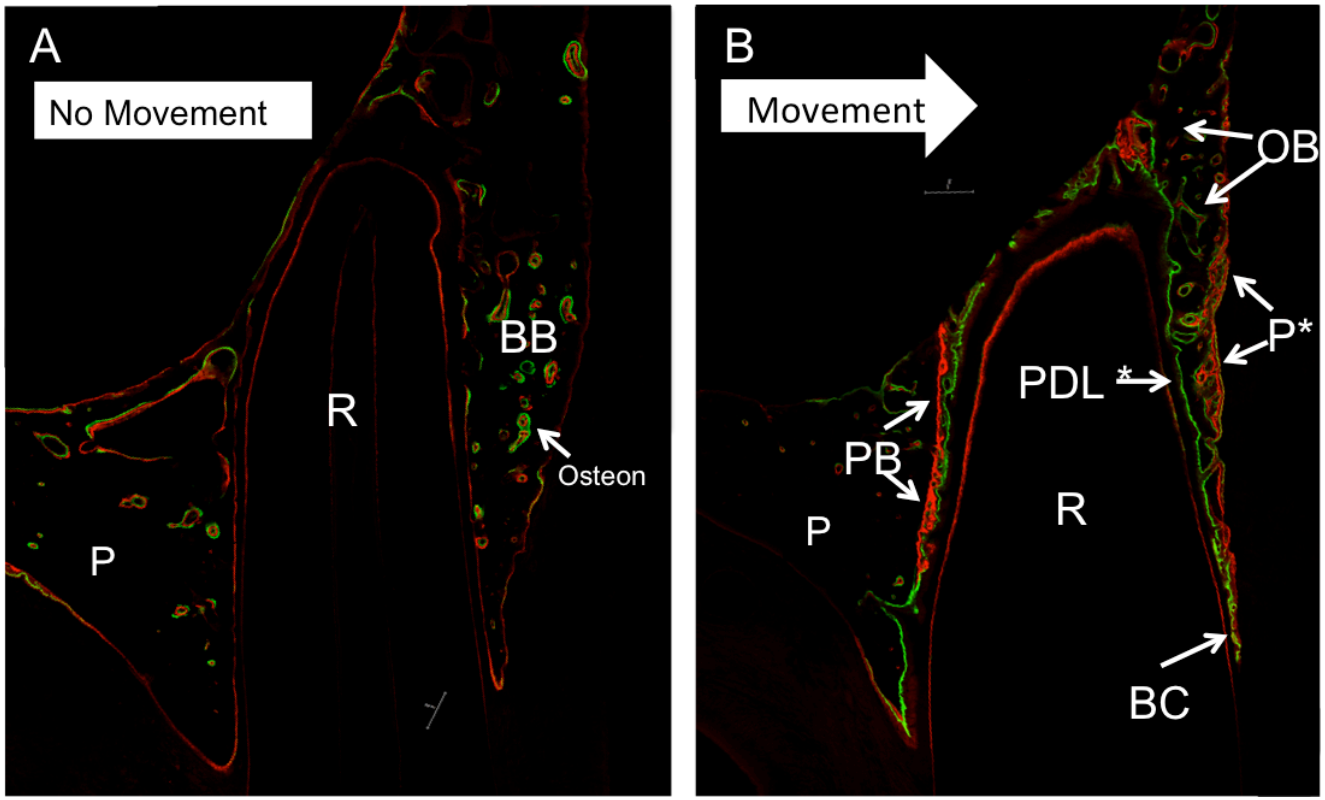

Figure 11. **Fluorescent Imaging**. Dog E longitudinal (coronal sections) fluorescent imaging of distal roots. **A**. Control demonstrates new bone within remodeling osteons of the alveolar bone. Calcein (green) and alizarin (red) labels were given at weeks 4, 6, and 8. Tooth movement was stopped at week 7. **B**. Experimental section shows new bone on both the periosteal and PDL surfaces of the buccal plate. The distinction between periosteal and PDL derived new bone is unclear near the crest. New bone is evident along the PDL side of the palatal bone. Similar thickness along the length indicates minimal tipping. (**P**= Palate, **BB**= buccal bone, **R**= root, **PB**= new bone along the PDL surface of the palatal bone, **PDL***= new bone along the PDL surface of buccal bone, **P***= new bone along the periosteal surface of buccal bone, **BC** = bone crest)

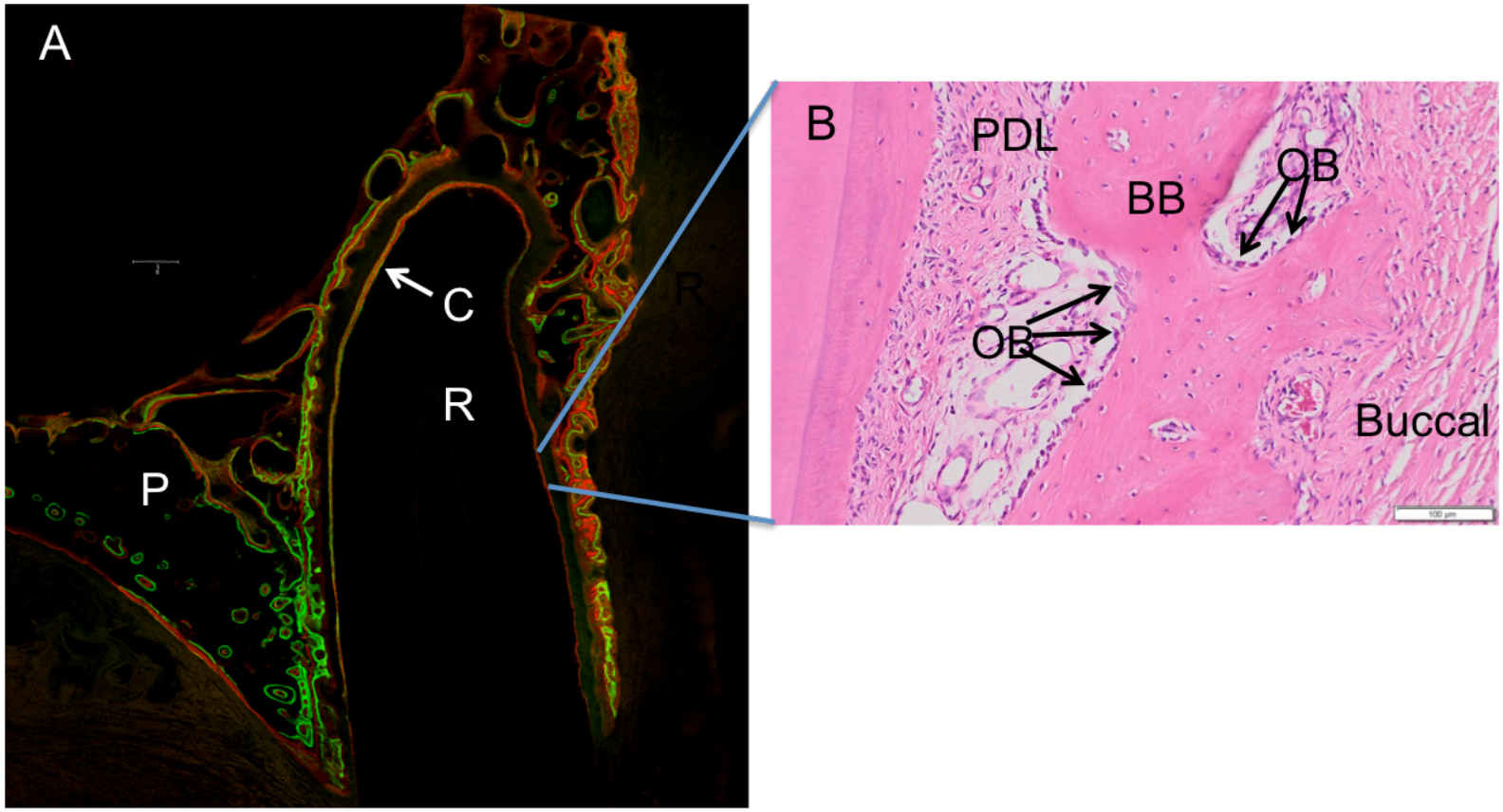

Figure 12. **Fluorescent Imaging and H&E Sections: Comparison**. Dog F (experimental) longitudinal sections. **A**. Fluorescent imaging of the mesial root demonstrates new bone laid down along periosteal and PDL surfaces of the buccal plate. Cementum also demonstrates green and red bands corresponding to new cementum. Corresponding H&E section of the distal root demonstrates active osteoblasts lining both the periosteal and PDL surfaces of the buccal plate, indicating new bone formation. (**P**= palatal bone, **C**= cementum, **R**=root, **BB**= buccal bone, **OB**= osteoblasts)

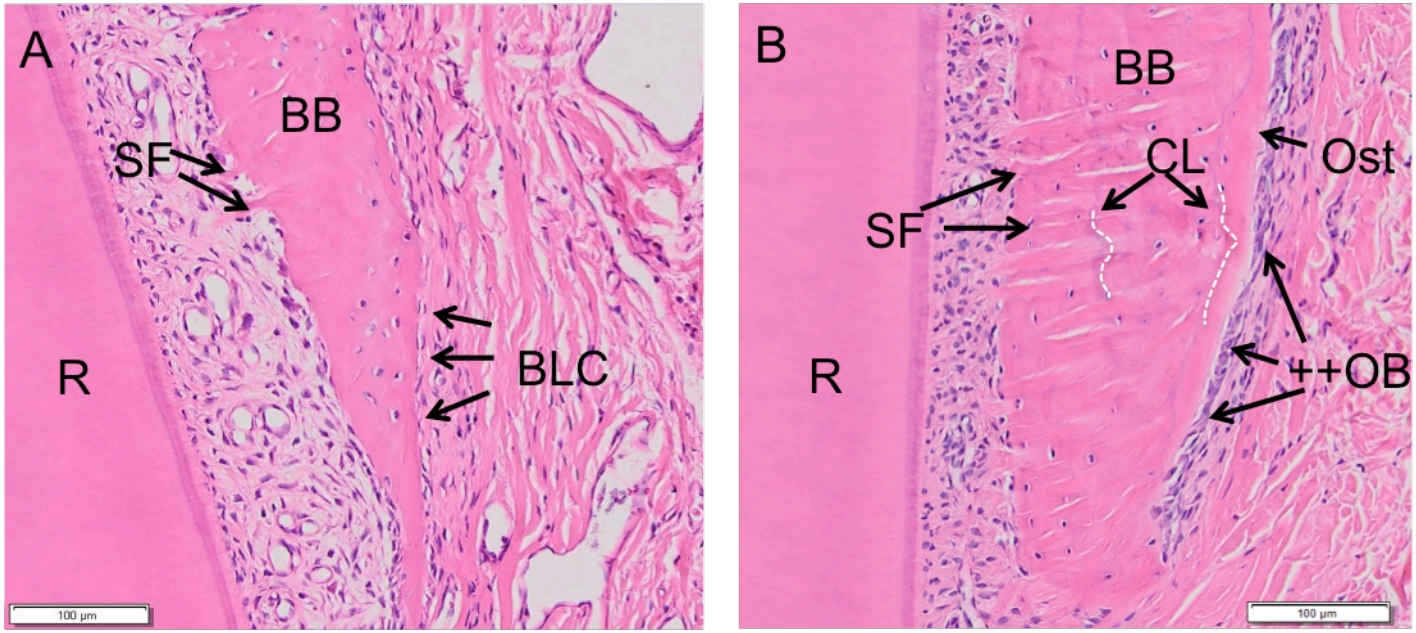

Figure 13. **H&E Sections**. Crestal bone of the buccal plate. **A**. Dog E control demonstrates bone lining cells (inactive osteoblasts) along periosteal surface. Absence of cement lines, as well as thinner and less numerous Sharpey's fibers are noted. **B**. Dog B experimental sample demonstrates active osteoblasts lining the periosteal surface of the buccal plate. Cement lines are present and Sharpey's fibers are more numerous and less organized. (**BLC**= bone lining cells, **BB**= buccal bone, **SF** = Sharpey's fibers, **R**= root, **CL**= cement lines, ++**OB**= abundant osteoblasts, **Ost**= osteoid)

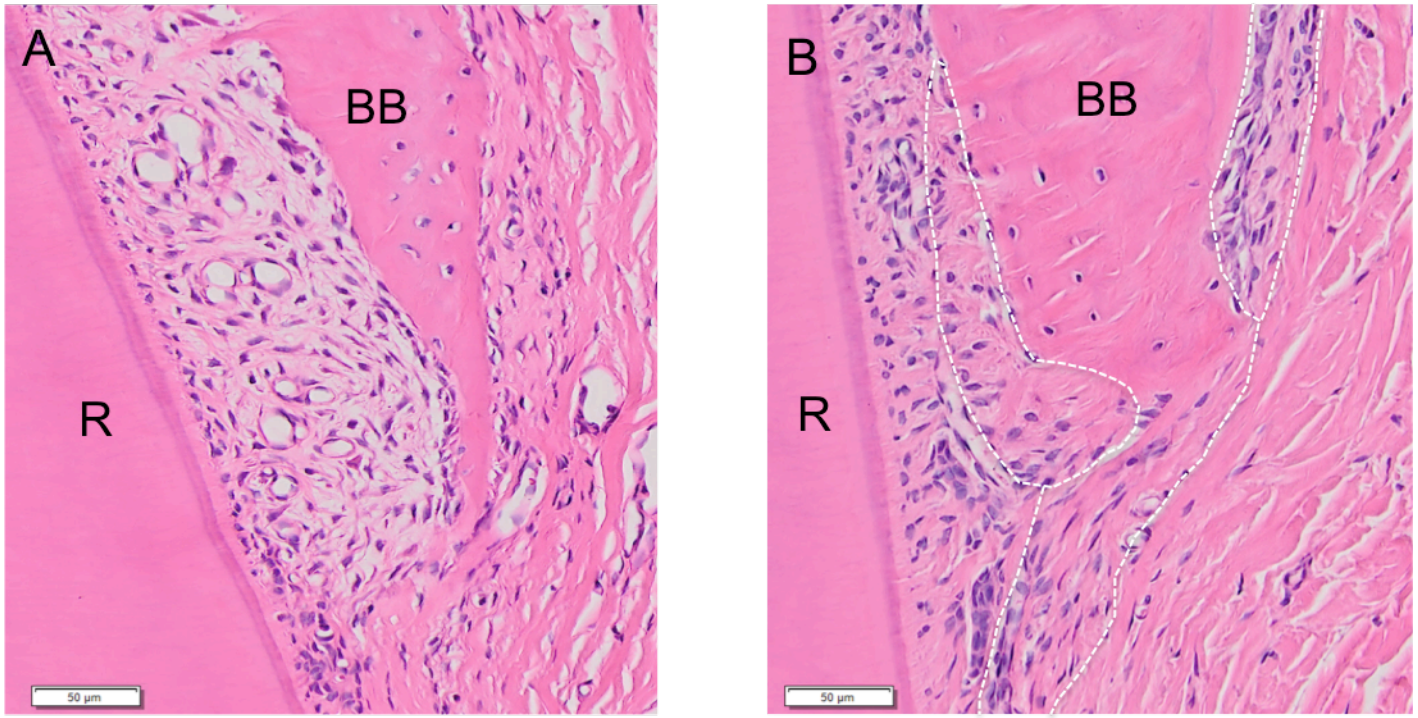

Figure 14. **H&E Sections: Bone-like Matrix**. H&E sections of crestal bone of the buccal plate. **A**. Control demonstrates bone lining cells (inactive osteoblasts) along periosteal surface. **B**. Experimental section demonstrates a bone-like matrix on the periosteal and PDL surfaces of the buccal plate, as well as in a region extending coronally from the bone crest. (**BB**= buccal bone, **R**= root)

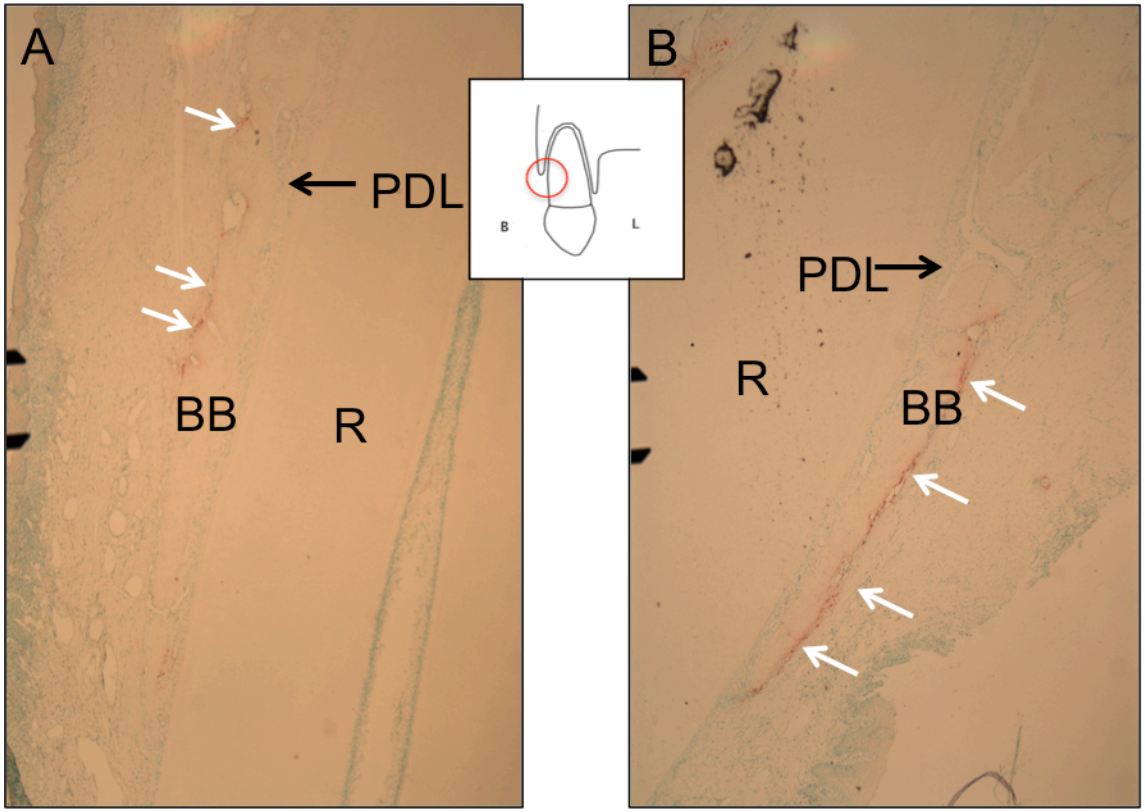

Figure 15. **TRAP Sections**. TRAP coronal sections of control (**A**) and experimental (**B**) teeth. Greater TRAP activity (red staining, white arrows) is shown on the periosteal surface of the experimental tooth D compared to control tooth E. (**BB**= buccal bone, **R**= root)

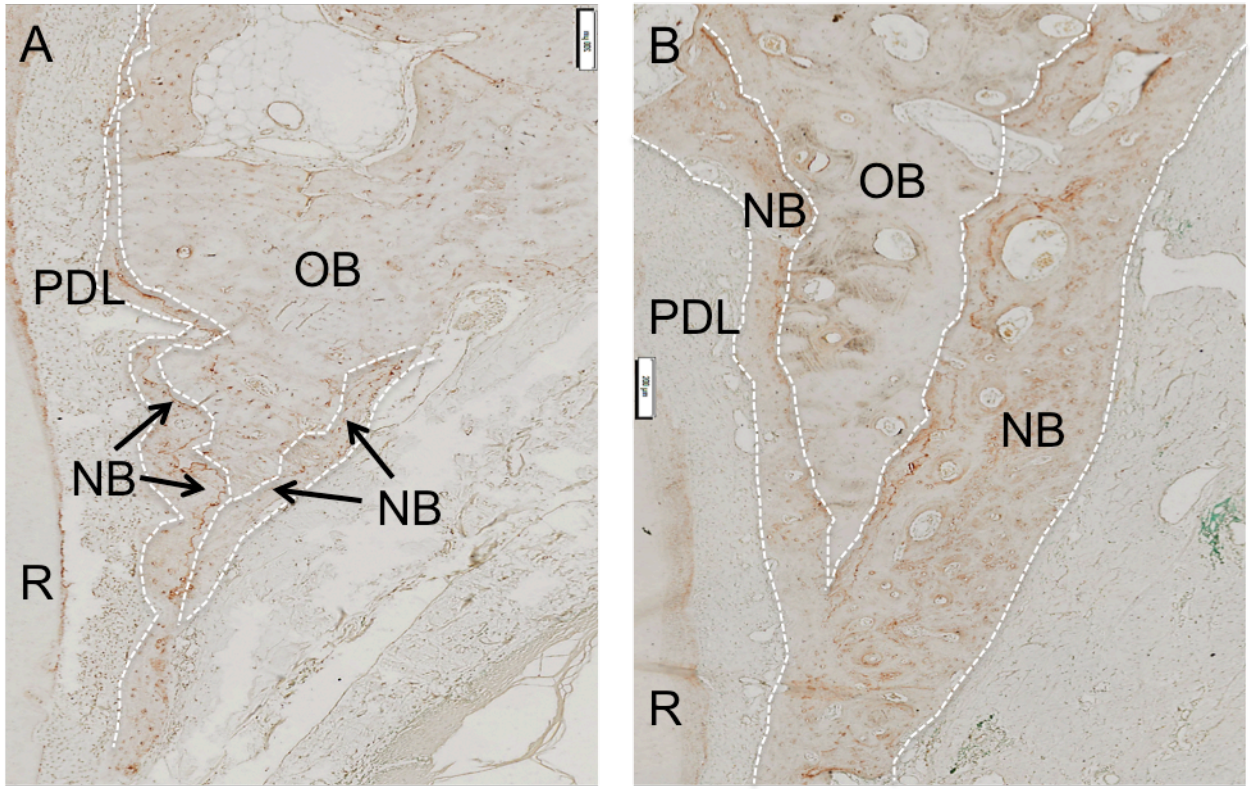

Figure 16. **BSP Immunostaining Sections**. Control (**A**) and experimental (**B**) teeth. Note a thick band of new bone formation on the buccal plate of the experimental (Dog D) sample relative to a thinner band in the control sample (Dog G). (**R**=root, **NB**= new bone, **OB**= old bone)